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MICROSCOPY

IN THE SERVICE OF MAN

By

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PREFACE

It is the aim of this book to give the general reader some account of the nature and scope of microscopical investigation. Save that a chapter is devoted to a brief description of the microscope and its use, microscopic technique is subordinated to the results of investigation and their value to man.

R. M. N.

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MICROSCOPY

CHAPTER I

THE MODERN MICROSCOPE AND ITS USE

WHAT is microscopy? It is the investigation of the very small by means of the microscope. What is a microscope?

It is popularly held that a microscope is an instrument for magnifying small things, but we shall appreciate the *rôle* of the microscope far better if we say that it is an instrument by means of which we are enabled to see minute detail which would otherwise be invisible. It is little use magnifying a thing if we do not see more in it; and accordingly, a well-equipped microscope is judged, not as to how many times it will magnify a specimen, but as to how much fine detail it will reveal when magnifying it. Such separating out or distinguishing detail is called *resolving*. Resolution and magnification should be carefully distinguished. They do not necessarily go hand

in hand. Some of the simple microscopes of the early microscopists magnified in a most extraordinary way, but their defects of resolution were so grave that it is not to be wondered at that sometimes imagination stepped in where glasses failed.

Starting from very humble beginnings, the modern microscope is a very perfect instrument. Many different patterns are found, each at its best perhaps as good as any. We shall first describe in detail a typical modern microscope which is thoroughly efficient without being complicated by the multiplicity of luxurious accessories which are the perquisite and the delight of the accomplished microscopist.

Looking at a microscope, we may recognise a useful separation into two sets of parts, *optical* and *mechanical*, or, if we like, glass parts and brass parts. The glass parts do the work, so to speak, of the instrument, the brass parts make possible their working.

The total height of the instrument on Fig. 1 is about a foot. We begin with the optical parts. There are three of these, eyepiece, objective and condenser, naming from the top downwards. (It will be noticed that in Fig. 1 there are two objectives shown, one in position, the other ready to be used when needed.)

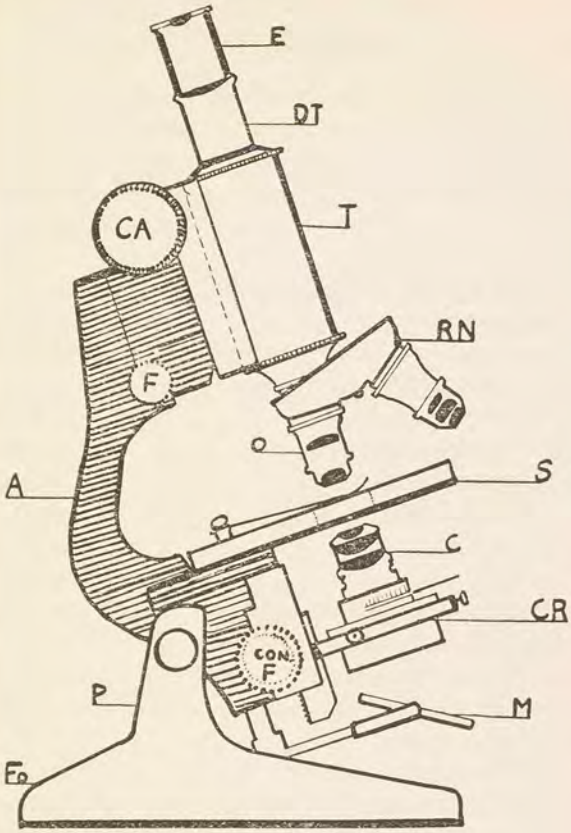


FIG. 1.—A TYPICAL MODERN MICROSCOPE.

1. *Optical parts* :—E. eyepiece. O. objective. C. condenser.
2. *Stand* :—Fo. foot. P. pillar. A. arm. T. tube. DT. drawtube. RN. revolving nosepiece. S. stage (with clip for specimen). CR. condenser carrier, with centring screws. M. mirror.

Controlling screws :—CA. coarse adjustment. F. fine adjustment. CON. F. condenser focusing screw

The objective is fitted at the lower end of a short wide *tube*, into the top of which a smaller tube glides. The eyepiece is in the top of this tube. Below the objective is the condenser, fitted in a carrier. These three—eyepiece, objective, condenser—are in perfect alignment. That is to say, their optical axes coincide.

So much for the glass parts. The rest of the microscope—the mechanical parts—is summed up in one word, *the stand*. There is a heavy base with a short pillar rising from it. The pillar carries a stout *arm*, which is inclinable through a considerable angle and which can be clamped firmly in any desired position.

The Arm.—(1) On the upper part of the arm the tube moves up and down by rack and pinion: the movement controlled by the ample screw head termed the *coarse adjustment*. Below the coarse adjustment is a smaller screw head, the *fine adjustment*, for the most delicate control.

(2) Below the region of the tube a *stage* is set firmly in the arm. A hole in the centre of the stage permits light to pass uninterrupted through the three optical parts.

(3) Below the stage, on the lower part of the arm is a ring carrier, moving up and down by rack and pinion, in which the condenser is carried.

(4) Fixed to a "tail piece" right at the lower end of the arm is a *mirror* swinging in gimbals: freely adjustable to any angle or direction to catch the light and reflect it up into the condenser.

How it Works.—Let us have it plainly. There is a specimen on the stage, a human eye applied to the eyepiece. Light strikes the mirror, and is reflected into the condenser, which bends it on the specimen. The specimen is thus brightly lit. The objective forms a magnified picture of the specimen far up the tube. This picture is caught up by the eyepiece, remagnified and passed into the eye.

Summarising, our microscope has a base and pillar supporting an arm which carries three sets of fittings; (1) the tube with eyepiece and objective; (2) the stage; (3) the sub-stage apparatus (a convenient term for the condenser and its accessories). It will now be necessary to look a little more closely at the details of these three parts.

The Tube.—We have noticed there is a smaller eyepiece-carrying tube sliding in the main tube. Why is this? It allows the distance between eyepiece and objective to be varied by sliding the draw tube. There are several advantages in this, but the main one is that all objectives are constructed to work

best with a definite "tube length." This is not a matter of great moment with low-power objectives, but is very important with high powers.

The eyepiece may have a magnifying power of from two to fifty times. By far the com-

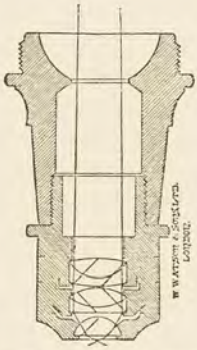


FIG. 2.—STRUCTURE OF A MICROSCOPE OBJECTIVE SHOWING SERIES OF LENSES.

monest powers for eyepieces are × 6, × 8, × 10.

The objective: the tube in Fig. 1 is fitted with an almost universal "revolving nosepiece." This allows 2 (or even 3 or 4) different objectives to be kept mounted on the microscope and used in rapid succession by being simply revolved into line. These objectives

are not simple lenses but are built up of a number of lenses fitted together. Each of them bears two index numbers, signifying the *focal length* and the *numerical aperture*. These are of great importance.

Focal Length.—The focal length is an index of the *magnifying power* of the objective. It is engraved on the objective as a measurement in inches or millimetres, and it is customary to speak of a 1 in. objective, a $\frac{2}{3}$ in. objective and so on. When the microscope is correctly adjusted to give a clear picture, the distance from the specimen to the middle of the series of lenses that constitute the objective is the focal length of that objective. Now the greater the magnifying power of a lens the nearer must it be placed to the specimen to get a clear picture. So that the shorter the focal length the greater the magnification by the objective. Thus a 1 in. objective will magnify 10 times, a $\frac{2}{3}$ in. 15 times, a $\frac{1}{6}$ in. 60 times, and a $\frac{1}{12}$ in. 120 times. (N.B.—There is a second magnification by the eyepiece.)

The microscope in Fig. 1 is fitted with a $\frac{2}{3}$ in. and a $\frac{1}{6}$ in. objectives, two of the most widely used powers, and very commonly referred to as the "low power" and "high power" respectively.

Numerical Aperture.—The other index

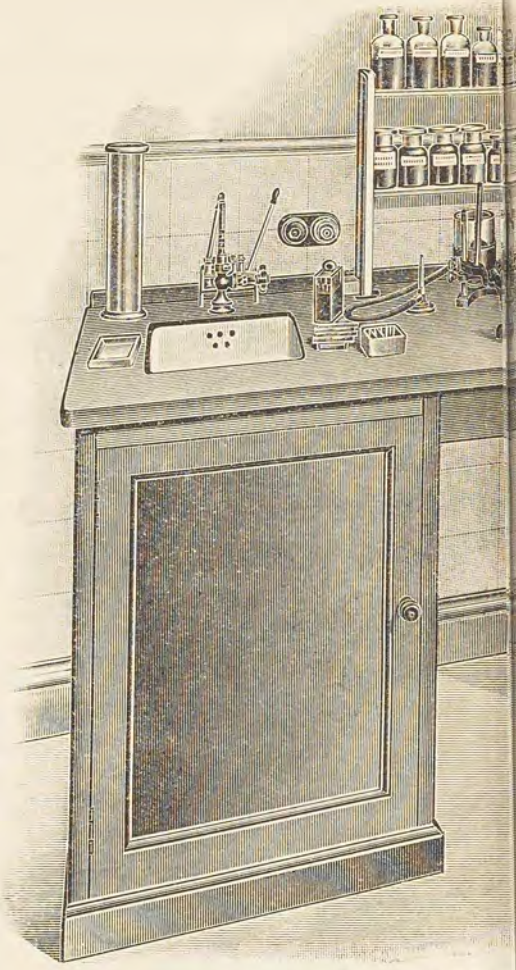
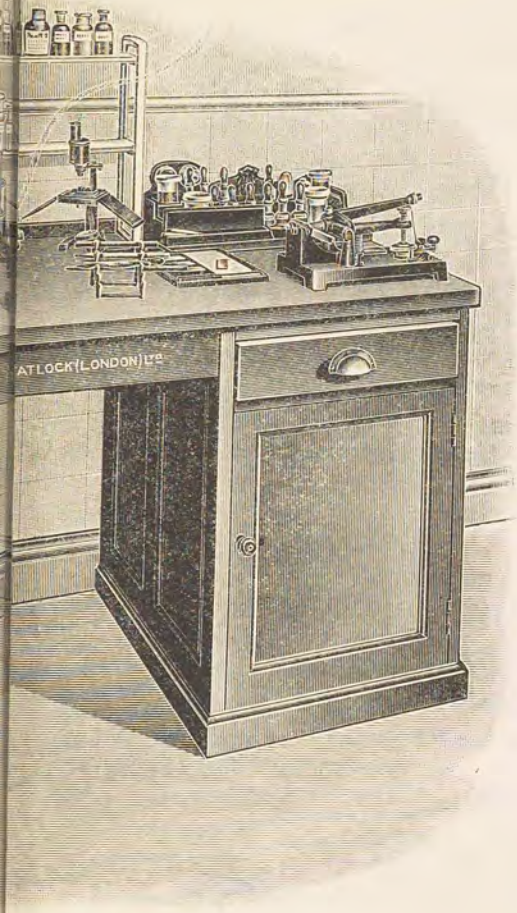


FIG. 3.—A MICRO



T'S WORK BENCH.

figure is the Numerical Aperture, usually written, N.A. The numerical aperture is for practical purposes an index of the *resolving power* of the objective. Typical N.A.s are

For a $\frac{2}{3}$ in. objective N.A. 0·30
$\frac{1}{6}$ in. objective N.A. 0·85
$\frac{1}{12}$ in. objective N.A. 1·37

The higher the N.A., the greater the amount of fine detail resolved : so that in objectives of any given focal length, the best one is the one with the highest N.A.—as is usually indicated by the price. The question of N.A. is a very important matter with $\frac{1}{12}$ in. objectives. These have the highest resolving powers and every little extra is valuable. We may have three of these objectives all approximately of the same magnifying power, with N.A. 1·25, 1·32, 1·37, respectively. The latter will show up much finer detail than the first and may be twice as costly.

We have then valuable information on the objective in these two figures, for focal length and numerical aperture, showing magnifying power and resolving power.

Achromatic and Apochromatic Objectives.—The old-time lenses showed a number of optical defects such as distortion of one

kind or another, colour fringes, etc. In the best *achromatic* objectives all these faults have been corrected—nearly. Apochromatic objectives, which are constructed in a different way we may consider perfection. They are costly, however, and achromatic objectives used intelligently answer almost every purpose.

THE STAGE.—The next set of fittings is the stage and in the illustration a plain stage is figured. It is large to accommodate dishes with specimens in fluid. For microscopic preparations mounted on a glass slip or slide, there are two clips to hold the slide in position. An additional fitment which is necessary in certain work and often desirable is a "mechanical stage." This when properly constructed, and preferably built into the microscope, permits of the specimen travelling from side to side, and backwards and forwards, by the turning of a screw or screws. The whole specimen can thus be systematically searched or examined with the certainty that no part has been overlooked.

THE CONDENSER.—The condenser is built of several lenses in the same way as the objective. It is held in a carrier which is controlled by a screwhead prominent below the stage. The mirror directs light up into the condenser,

which bends the light on to the specimen. The amount of light coming into the condenser is controlled by an iris diaphragm arranged just below it.

The condenser is a very important part of the microscope, as on its intelligent use depends how much even the best objectives will achieve in everyday working. To appreciate this we must recall that N.A. indicates the resolving power of an objective. Now the resolving power of an objective depends on the *amount of light that can pass through it* from every point on the specimen. In other words, the resolution is bound up with the efficiency of the condenser in illuminating the specimen. What are the hall-marks of an efficient condenser? There are two. It must permit the passage of a large amount of light and it must bring that light to a very exact point on the specimen. To do this, a condenser must be constructed free from all optical defects, that is, it must be as perfect as an achromatic objective of high N.A. This cannot be too plainly laid down. Such a condenser is a necessity for all serious work involving high-power objectives, and it is advisable always. The beginner frequently dispenses with a condenser, if low-power work is being contemplated, and no great harm is done, but the

optimum illumination of the specimen and hence the best resolution cannot be attained without a fully corrected achromatic condenser of adequate light-passing power.

From this brief description of a modern microscope, necessarily a little technical, we pass to the manner of using the microscope and the manner of preparing specimens for examination : the latter an aspect of microscopy which has contributed in no small degree to the results which have been achieved through the microscope. But before we do this, it is necessary to refer to the nature of the light used in making observations by means of the microscope.

LIGHT.—There are two things which must be under control, namely, the strength of the light and the colour. This seems to exclude daylight. While there is no doubt that daylight is sometimes very convenient for casual observations with low powers, it is generally so *diffuse* that it is difficult to obtain a parallel beam of light, and it is very desirable that there should be a parallel beam of light falling on the mirror. Further, daylight is variable in intensity and also not powerful enough for a great deal of high-power work. It becomes necessary, then, to select something more reliable. A great favourite with many

microscopists is a small oil lamp with a flat wick, but in general some form of electric luminant is used. This varies from a bulb of the usual household type, with a frosted or opal globe to an arc lamp of small size. In general, the stronger the light is, the better, for it is easy to control the intensity by interposing a neutral-tinted screen between lamp and microscope. In front of the lamp an accessory lens is placed from which a parallel beam of light falls on to the mirror. In recent years, a most useful form of illuminant has been introduced where a small electric bulb with accessory lens complete is fitted on to the tail of the microscope arm instead of a mirror, and shines directly up into the condenser.

The colour of the light is a point of considerable importance in several branches of work. Knowing the kind of light given off by the lamp we are using, we can obtain light of almost any wave-length, *i.e.*, of almost any colour, by placing a screen of the right tint in front of it. Screens are either liquid (contained in a glass trough) or made of coloured gelatine, tested by the spectroscope, and sandwiched between two plates of glass. Indispensable in photomicrography, such screens are a great asset in visual work. To give a simple example : if some of the minute uni-

cellular creatures found in stagnant pools are being examined in white light, many of them may appear of a pale greenish tint. They are often very transparent and details are made out with difficulty in ordinary white light. Now, by placing a red screen (for red is the complementary colour to green) in position, and examining them again, the green appears deep black against the red light; and we secure greater contrast in the details of structure. On the other hand, with a very dense green specimen, the use of a green screen will reduce contrast. The specimen will appear more transparent and fine detail can be made out that had hitherto been obscured. Such selection of the right colour of light for the purpose in view is very useful in the examination of stained or coloured plant and animal tissues.

This use of coloured light must be carefully distinguished from the use of polarised light, which we shall refer to in Chapter IV, and which, as we shall see, is a very different thing.

Using the Microscope.—Such then is our instrument. How is it used?

To begin with, the specimen we are to examine must be suitably prepared. It may be some small creature in a watch-glass or

other glass vessel of fluid; in which case the arm of the microscope must be set upright; or it may be a section of plant or animal tissue permanently mounted on a slip of glass 3 in. × 1 in. termed a microscope slide. We shall illustrate the preparation of such specimens later. For the time being, we propose to examine a preparation mounted on a microscope slide.

It has been said that for low magnifications it is possible to obtain fair results without the use of a sub-stage condenser. For simplicity's sake, we may first indicate the steps in the using of the microscope with the condenser removed, and discuss the use of the condenser thereafter.

The microscope is placed opposite the illuminant; the low power ($\frac{2}{3}$ in.) revolved into position, and the arm inclined to a comfortable angle for working. The first thing to adjust is the tube length. On the draw tube is engraved a scale of millimetres, and every maker of objectives indicates the tube length for which his objectives are made: it is often engraved on them. Making due allowance then for the fact that a revolving nosepiece lengthens tube length by 15 mm., the draw tube should be set to the appropriate length by the scale.

The second step is to apply the eye to the eyepiece and manipulate the mirror to get a good "field of light." This manipulation of the mirror is best done by the first finger and thumb, both hands being employed. The mirror being in gimbals, the flat side of it can readily be turned in the right direction to catch the light and then tilted to the correct angle to throw the beam up through the stage. When the mirror is properly adjusted, the circular field seen on looking down the microscope will be brightly and uniformly lit.

By turning the coarse adjustment, the objective is brought down to within a short distance, say $\frac{1}{4}$ in., of the stage, and the specimen-slide taken up. The slide is placed on the stage as near as may be in the middle, the actual specimen being about the centre of the stage opening. Looking down the microscope, the slide is moved about gently with the fingers until the blurred image of the object comes into the field of view. The slide is then clipped in place.

The next step is to focus. The objective is lowered by the coarse adjustment till it nearly touches the slide, the eye is applied to the eyepiece and the coarse adjustment slowly turned back till the image shows clear and sharp. A final touch of the fine adjustment

(rarely needed with low powers) produces critical sharpness.

As a routine method of procedure, we must follow the order (1) Tube length; (2) Mirror; (3) Specimen; (4) Focus.

It should be pointed out that the image given by the microscope is an inverted image. So that in cases where it is desirable to view the specimen " right side up," the slide should be placed " upside down " on the stage.

Using the Condenser.—For efficient high-power observation we must replace the condenser and adjust it. The specimen is first focussed with as good a light as possible from the mirror, and we proceed to centre the condenser. It is a step that should be done with care, as it is extremely important that the light rays emerging from the condenser should converge toward the exact centre of the field of view and thus illuminate it evenly. Below the condenser is an iris diaphragm. This is shut down till its opening is a small pinhole. The condenser is lowered by means of its controlling screw, and looking down the microscope an image of this pinhole is seen in the field of view. If it is in the centre, well and good; if not, the centring screws of the condenser must be manipulated till it is.

With the condenser central, the iris dia-

phragm is opened out again and we proceed to focus the condenser, by its controlling screw. The condenser is moved up (a very gentle movement is needed) till the image of the *illuminant* is seen in the field of view. The very slightest lowering of the condenser thereafter gives the best possible illumination.

With the condenser throwing a strong and even light on the specimen, the high-power lens is revolved into position, the eyepiece taken out and the *back* of the objective examined. It will probably be found flooded with light. Closing of the sub-stage diaphragm cuts off superfluous light; and it should be closed till about three-quarters of the back of the objective is bright, the periphery appearing dark. All that remains is to replace the eyepiece and focus the high power.

This way of using the microscope with light passing through the specimen is termed working with *transmitted light*. The only other method of illumination we need mention here, for it is especially used by those microscopists who, for one purpose or another, use chiefly low powers—on insects and other largish specimens—is *reflected light*. Here no light comes up through the specimen and the mirror is not used. A beam of light from the lamp is converged directly on to the specimen

as it lies on the stage; so that it is brightly lighted while the rest of the field remains more or less dull.

PREPARATION OF SPECIMENS FOR EXAMINATION.—The whole aim and object of "pre-

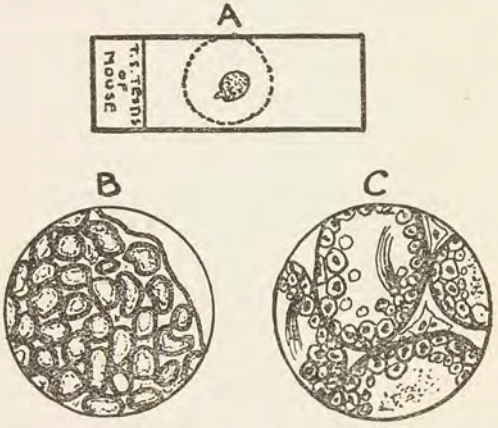


FIG. 4.

A. A microscopic specimen mounted on glass slide.
B. Part of the specimen under a low power.
C. Part of B under high power.

paring" specimens is to enable us to learn more about them: by mounting them in the most convenient fashion, by colouring them with stains or by cutting them into thin slices and afterwards staining these in two

or more colours. Further, some of the specimens may be for temporary use; others it will be desirable to prepare in such a way that they may be kept permanently. Let us illustrate.

A. Mounting for examination: some specimens like eggs of fishes, and small embryos, from their very nature must be examined in fluids in a dish. Smaller specimens are usually mounted on a 3 in. × 1 in. glass slide, and covered with a very thin glass *coverslip*.

B. Temporary mounts: a drop of pond water containing minute organisms or small living eggs may be placed in a slightly hollowed-out slide, covered with a coverslip, and the creatures examined alive: or a fragment of torn-up tissue on a slide may be covered with glycerine and a coverslip applied. This style of preparation may often be kept permanently by sealing the edges of the coverslip with gold size followed by cement.

C. Dry mounts: a small insect or the head of a spider may be fixed to the slide by a touch of adhesive. In this case, owing to the thickness of the specimen a little wall of glass or cardboard is made round it to support the coverslip and avoid crushing the specimen. This makes quite a durable preparation.

D. Permanent preparations; usually involving the staining of the specimen. Most

tissues and many small organisms cannot be thoroughly studied in a fresh condition by transmitted light because they are colourless or nearly so, because they soon spoil and because their texture does not permit of their being easily cut into slices thin enough to examine. It is necessary, therefore, to immerse them in stains which act on the tissues in such a way that the different elements in the tissues are shown up, to use special means for obtaining very thin slices, and to mount the preparation in permanent and durable fashion.

The most common way of permanently mounting specimens is in Canada balsam, a resinous medium intolerant of moisture, hence all moisture in the specimen must be got rid of. Before this is done, however, the first and most important step must be taken, viz., killing the tissue in such a way as to avoid any changes in its structure. This is called *fixing* the specimen. Full details of fixing fluids, which are many and various, may be found in text-books (see Bibliography) and there is no need to go into them here. As an example of a very widely used one, we may instance Bouin's Fluid, composed of 75 parts by volume of saturated watery solution of picric acid, 25 parts of formalin and 5 parts

of acetic acid, measured exactly. After fixation, the fixing fluid is washed out (with 70 per cent. alcohol in the case of Bouin's Fluid) and the specimen is ready for staining. When staining is complete all remaining moisture is removed by *dehydration* in baths of alcohol of gradually increasing strength till the specimen is saturated with pure (absolute) alcohol. Xylol is substituted for the pure alcohol and *clearing* takes place. The opaque specimen gradually becomes beautifully translucent and may then be removed to a microscope slide, covered with a solution of Canada balsam and a coverslip and allowed to dry.

There is great variety in the details of the various steps in the process, and it may be well to indicate the actual operations performed in making a preparation of a piece of animal tissue, a frequent procedure in numberless laboratories, explaining at the same time how the thin slices or sections necessary are obtained.

A small piece of the tissue, perhaps a piece of lung, is removed from the newly dead animal, fixed overnight in Bouin's Fluid and placed for a day in 70 per cent. alcohol. This is replaced on successive days by 90 per cent. and pure alcohol.

To enable thin sections to be made, the tissue has to be embedded in melted paraffin wax, which hardens into a solid mass. Sections are then cut through this block of paraffin, tissue and all, by means of a mechanical contrivance called a microtome (see Fig. 5). If the paraffin block containing the tissue is to be quite homogeneous, the tissue must be saturated with the melted paraffin. As alcohol and paraffin do not mix, the alcohol-laden tissue is first placed in xylol, which is a solvent of paraffin. Saturating with paraffin follows for several hours, and then paraffin and tissue are poured into a mould, which is quickly cooled and the block turned out. The block is trimmed, adjusted on the holder of the microtome and a succession of thin sections cut by moving the microtome handle. Such slices may be $\frac{1}{2500}$ in. thick or thinner.

The sections are flattened out, placed on microscope slides smeared with adhesive and left for the adhesive to set. They are now ready to be stained.

The choice of stains, watery or alcoholic, is considerable : and on the nature of the stain depends to some extent the details of procedure. A widely used staining method is Hæmalum (in watery solution) followed by Eosin (in alcohol).

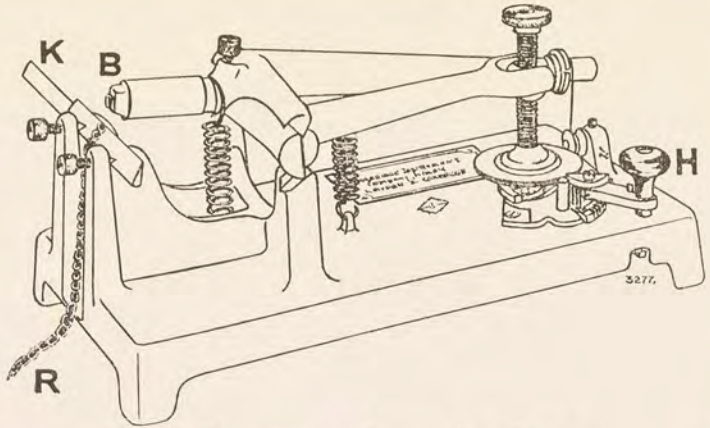


FIG. 5.—MICROTOME FOR CUTTING THIN SECTIONS.

B. Paraffin block with tissue. K. Knife. H. Handle. R. "Ribbon" of cut sections.

B

The paraffin is first removed by immersing the slide in a small jar of xylol. Pure alcohol removes the xylol and the slide with its section or sections is taken through a series of alcohols of descending strength and well washed in water. Staining in hæmalum for some minutes follows. From time to time the preparation is examined under the microscope till it is seen that certain elements —the nuclei—are stained a deep purplish-black, the remainder being faintly tinged. After rinsing in water, the slide goes through successive jars of alcohol of increasing strength, till from 90 per cent. alcohol it passes into a solution of eosin in 90 per cent. alcohol, a powerful red stain. After a minute or two in this, it will almost certainly be overstained. The superfluous colour is therefore extracted by means of fresh alcohol till the microscope shows a picture wherein darkly stained nuclei stand out on a pale pink background. Clearing in xylol, a drop of Canada balsam and a coverslip end the procedure.

Besides embedding in paraffin, there are various other methods of obtaining sections. Satisfactory sections of plant stems and similar specimens can often be cut by hand with a sharp razor, or embedded for cutting in

a piece of hollowed-out carrot. Very brittle objects are best embedded in celloidin. Rapid sections of newly fixed or even quite fresh tissue may be made by a freezing microtome, where the tissue is frozen hard enough to enable fairly thin sections to be cut. This method is much used in pathological work where speed may be an important factor.

There are innumerable special methods of staining both animal, plant and other specimens. Some are extremely complicated, but each and all have one end in view: to bring out the details of structure in the specimen and so facilitate their interpretation. Fascinating though the technicalities of specimen mounting be, it must never be forgotten that they are but a means to an end.

CHAPTER II

MICROSCOPY

From the time when the microscope in its early form emerged into general use, it has been a centre of attraction to many different folk drawn to it on different accounts. To some it has been the provider of "microscopic curiosities," to others a source of genuine pleasure and recreation; others, again, have found it a revealer of knowledge to mankind in more serious inquiries. While we are concerned in this book chiefly with the more mature results of microscopical investigation in which the microscope has proved a valuable tool to workers in different branches of science, we wish here to refer to the lighter side of microscopy, where the microscope itself forms the centre of a variety of interests. Such was the microscopy of many of the earlier microscopists. What is necessary, says one of them, but "good Glasses, good Eyes, a little Practice and a common Understanding, to

distinguish what is seen; and a Love of Truth, to give a faithful Account thereof"?

It may be well to point out that our description of a modern microscope in the preceding chapter referred to a style of instrument such as is widely used to-day in laboratories; but that from the point of view of recreation, great pleasure may be got at first from quite old-fashioned types of microscope, or even from a good pocket lens. Provided they are in good working order, such survivals are not to be despised. Frequently they do all that is required, and often pave the way for a more efficient modern instrument. Further, a very considerable variety of interesting studies is possible without need for much equipment in the way of chemical reagents. The fascination of such studies is evidenced by such statements as this: "To the owner of a microscope the whole aspect of the world alters; it becomes vaster and entirely new, filled with countless numbers of most beautifully formed animals and plants, very different from those to which he has been accustomed, and concerning whose existence he has hitherto been entirely ignorant."

We may distinguish three levels of micro-

scopy viewed as a recreation. The first of these is the examining of what may, for want of a better name, be called objects of general interest, the inspection of which is prompted by an interested curiosity. Under this head come all kinds of specimens, animal, plant, mineral and others. Common insects, eggs and wing-scales of butterflies, hairs, feathers, the stings of bees, fleas, cheese mites, yeast, mould, drops of muddy water teeming with strange life, pollen grains, the pollen baskets of bees, seeds, leaf pores, iridescent wing-cases of beetles, fragments of granite and opal, shot-silk fabrics, and a long list more.

One of the most beautiful sights of the microscope is the brilliant coloration of the wings of many butterflies or of a bit of the shining feather of a peacock, seen by reflected light under a low power. The colours of animals in general are due either to pigments or to physical coloration, or to both these being present together. This latter is the case in the peacock's feather and the butterfly's wing. The wing is covered with a multitude of minute scales, each fitted into place and overlapping one another like slates in a roof. Their delicate sculpturing produces interference colours which vastly enhance the pigmentary

coloration. Apart from the wings, the head of the butterfly is interesting, especially the long coiled sucking tube or proboscis so finely adapted for dipping into flowers.

The peacock's feather tempts to an examination of other feathers and the detail of the broad vane of the feather is revealed. The strands or barbs which make up the vane are webbed together to form a light and resilient structure. The barbs bear on either side branches or outgrowths—the barbules. Barbules of adjacent barbs interlace and from each barbule smaller outgrowths—barbicels—arise. Many of these barbicels carry minute hooklets which interlock with adjoining barbules, and so a springy fabric is built up suitable to beat the air.

There is no commoner object in summer than the housefly, which does not bite, as many people suppose. Perhaps its most striking characteristic under the microscope is its "hairiness," the hairs formed of tough chitin. We see at once the potentialities of the fly as a microbe carrier. The delicate jointed legs end each in a strong claw with a little pad or pulvillus beneath, which aids the fly in its familiar feats of running on impossible surfaces. Three simple eyes lie on the top of the

head between the great compound eyes, each with its four thousand facets, each facet the outer surface of a unit of the eye or ommatidium. The width of the head between the great eyes helps to indicate the sex of the fly. Then there is the mouth region. A soft sucking proboscis (often erroneously called a tongue) depends from the head ending in two soft lobes through which the fly sucks liquid food. There is no provision of any kind for solid food or for biting.

A microscopic specimen of a more uncommon kind is ambergris. Ambergris is found floating in the sea and originates as a concretion in the digestive organs of the sperm whale. Microscopic examination will reveal numbers of particles of horny material—chitin; part of the " pens " of cuttlefishes which, hunted at great depths, form part of the food of the toothed whales.

At a second level comes the microscope used to further an existing pastime. Gardening comes to mind at once: green fly and other garden foes, the structure of leaves, the crystalline bodies in many plants, the sculpturings of pollen grains and seeds and the reproductive organs of plants.

The angler-microscopist takes a keen delight

in the study of aquatic insects, from which to prepare more cunningly fashioned dryflies to beguile the trout, and then finds further microscopic material in the tiny round worms in the food canal of the trout when basketed.

FIG. 6.—SALMON SCALE SHOWING RINGS OF GROWTH.

If he be a salmon fisherman, a fresh-run fish will provide those degenerate crustaceans termed fish lice, as well as silvery scales whereon he may read the life-story of the salmon. The salmon's scales under the microscope show concentric lines of growth, like the zones on a shell or the rings of trees. A

broad zone marks a summer of rich feeding and abundant growth. A narrow band the winter, when food is scarce and growth less rapid. Further, if the fish has spawned, there are indications of the wear and tear of the journey up-stream, resulting in a ragged edge to the scales, later surrounded by a new summer's growth. A fine spring salmon recorded by Mr. J. A. Hutton showed the record of the following history on its scales. It was hatched from an egg deposited in the winter of 1903, and spent the first two years of its life in the river where it was born. Then it went down to the sea in 1906, and remained there three years, feeding well and growing till the urge of reproduction prompted a return to the river in 1909, when it was captured.

A more contemplative pastime is that of bee-keeping. The bee-keeper learns much from his microscope. Especially does he search for evidence of the deadly infection of the breathing tubes of his bees by mites which betoken Isle of Wight disease.

An unusual linkage is microscopy and stamp collecting. A specially designed microscope of simple pattern has been designed for examining stamps, their details of design and

water-marks. The stamp is sandwiched between two microscope slides for examination : or the stage may be removed from the microscope and the stamps on large documents, which need to be spread out on the table, readily examined.

At a third level comes the microscopist who embarks on a definite line of study—the general microscopist merging into the scientific investigator. The attractions of pond life have always had great favour with users of the microscope; minute seashore life less so perhaps, but still of absorbing interest. Another large group find the study of lesser insect life an unending pleasure.

The water of a stagnant pond is always interesting at any time of the year. The waters and the floor of the pond swarm with living specks mostly on the lowest rung of life's ladder, many on the borderline between plants and animals. A scoop of muddy water provides an endless diversity of forms. In the spring, the numbers increase at an appalling rate. Most of these very simple organisms reproduce by dividing into two when their limit of growth is reached, and a calculation of the continued division, beginning with a single individual, soon runs into millions.

Professor Woodruff found that the slipper animalcule's rate of ordinary division was three times every 48 hours. Let us mention some of these minute creatures.

Perhaps first in point of charm should come the bell animalcule—*Vorticella*, so quaintly described by the old Dutch microscopist. A group of *Vorticellæ* moored to a fragment of duckweed is like a cluster of miniature lilies. A slender stalk with a contractile filament within it is surmounted by an upper part like a wine glass or an upturned bell. Round the rim of the bell is a fringe of flickering hair-like cilia. Within, closing the mouth of the bell, is a disc or lid in which is a food aperture. From the surrounding water, the cilia waft minute food particles. *Vorticella* is extraordinarily sensitive: a tap on the microscope table and the stalks shut down like a spiral spring with lightning rapidity, to straighten out by and by.

Then there are monads, sun-animalcules, and the spirally moving *Chilomonas*, *Euglena*, and the slipper animalcules, and *Volvox*. *Euglena* is a bright green creature—often spindle-shaped, moving by the lashing of a thread-like flagellum, with a prominent speck of bright orange-red pigment near the end

bearing the lash. The green is due to chlorophyll, the green colouring matter of plants.

Volvox is peculiarly interesting, as it is a colony of hundreds of individuals, each with

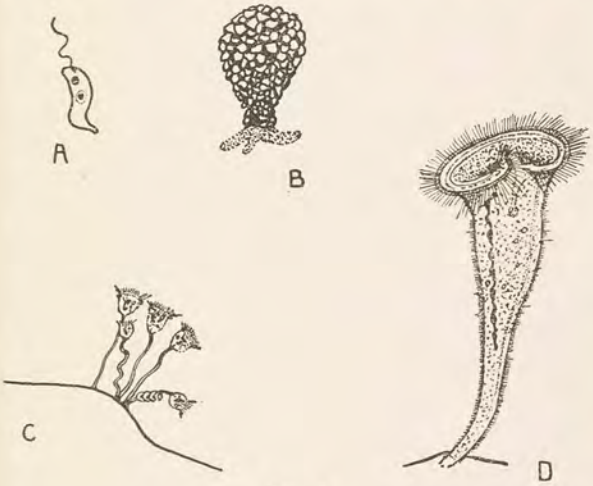


FIG. 7.

A. Euglena. B. Difflugia. C. Vorticella. D. Stentor.

two small flagella, grouped into a hollow ball, green with chlorophyll, with smaller balls—young colonies—inside it. By the movement of the flagella the green ball bowls along in the water. *Volvox* is remarkable for

frequently showing a division of labour amongst the individuals which compose it. Some of them are specially modified for reproduction.

A little higher comes the fresh-water polyp *Hydra*, green, brown or reddish-yellow in colour, a tiny double tube adhering by one end to aquatic plants, the other end conical with a mouth surrounded by half a dozen or more tentacles. Stinging cells stud the tentacles and kill or numb smaller fry, which are then drawn into the mouth. Frequently young individuals may be seen budding from the parent and the *Hydra* has an appearance in accord with its name.

The seashore is an equally rich collecting place, abundant in microscopic life: algæ, zoophytes, sponges, eggs of molluscs and fishes, very young stages of crabs, fish, and a host of other forms. Some of the delicate hydroids found on stones, shells and the like in still pools show a bright, glassy tunic extending over the whole of the stalk and branches, and expanding at the end of each branch to form a cup with a series of constrictions below it resembling a pile of rings. These cups house the polyps of the colony. Closed capsules—gonothecæ—occur here and there from which

from time to time minute swimming bells—medusoids—are shed out. These, after being detached, lead a free swimming life and eventually produce eggs which, when fertilised, give rise to ciliated planulæ which settle down

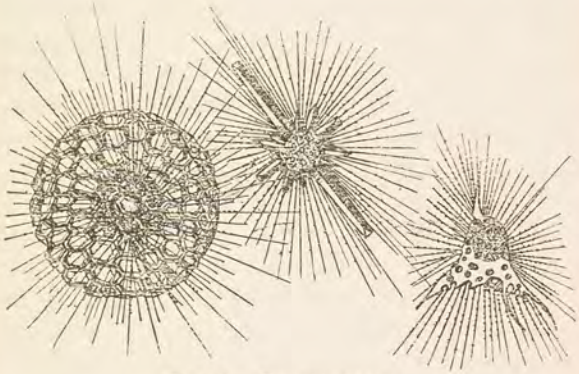


FIG. 8.—RADIOLARIANS.
(After Bütschli, *Protozoa.*)

on a rock and develop into new colonies of fixed polyps.

As microscopic objects of striking beauty, none are equal to foraminifera and radiolarians. These simplest animals have a wonderfully complex skeleton or shell: the foraminifera of lime, the radiolarians of flint.

The former creep about at the bottom on stalks and fronds of seaweeds and some float at the surface. Their shells form great tracts of calcareous ooze on the ocean floor, and those of bygone ages appear in our chalky rocks to-day. Radiolarians are more pelagic, found at all depths in the open sea; their very hard skeletons carpet the ocean floor at great depths, where limy shells of the foraminifera would be dissolved ere reaching the bottom. The architecture of these tiny shells shows endless variety of pattern—spirals, lattices, and stars—and viewed by reflected light on a black background they are marvellously beautiful.

Vying with foraminifera and radiolarians in variety and even exceeding them in delicacy of sculpture are diatoms, the most abundant living things in all the waters of the globe. "Diatomists" are among the most skilled users of microscopes. Diatoms are minute plants all of microscopic size, with a box-like case or shell of silica in two parts patterned in most exquisite forms. They form a staple food for nearly all small animals, which in their turn are eaten by larger animals; and so on till a "food chain" is formed. The flinty shells are almost indestructible and are often

found in guano, having passed successfully through the food canal of water fleas, young fishes, bigger fishes and sea birds. They form the well-known "diatomaceous earth." The great microscopist Ehrenberg estimated that the deposits of this diatomaceous earth in Bohemia contained 40 million diatom shells per cubic inch. From time to time they multiply in such numbers that the water in some reservoirs and lakes is of the consistency of soup.

Such general studies with the microscope as we have referred to bear many fruits, but they never fail to excite a feeling of wonder and convey a sense of beauty: wonder at the extraordinary diversity of minute life, beauty of form and fitness. The old microscopists loved to contrast man's handiwork and nature's; the crude point of the needle with the delicate sting of the bee, the threads of lace with the exquisite gossamer of spiders. Many a fretted diatom is the rose window of a cathedral in miniature.

We turn from the pleasures of the microscope to the fruits of microscopy. It is needless to point out that the microscope is one of the most valuable of scientific tools,

associated with discoveries famous in the history of science and of paramount importance to mankind. What sort of results have been forthcoming from the use of the microscope in different fields? How have they benefited mankind? How are these results obtained? We seek to convey some answer to these questions by illustrating the service of microscopy to man in science, industry and human welfare.

So many and so varied have been the benefits direct and indirect of the introduction of the microscope that it is difficult in these knowledgeable days to recall the existing state of affairs—barely three centuries ago—before microscopy had begun to contribute its quota to the sum of human knowledge and the relief of man's estate. No one was aware of the enormous world of minute creatures which surround us on every side, and which include so many organisms of vital importance to man's well-being: such as the microbes of malaria, sleeping sickness, syphilis and the like. The early life-histories of even the larger animals were wrapped in mystery. The studies of heredity and development were buried in superstition. The study of living structure was largely confined to what we

now term " gross anatomy " : histology, or minute anatomy had not even begun. A bodily tissue was discussed by its outward appearance to the eye, by its weight or smell. Blood was a red vital liquid, not a fluid tissue of red blood-cells and six or seven kinds of white blood-cells floating in plasma. The cellular structure of animals and plants and all that this entails was completely hidden. Even after the invention of microscopes, two hundred years were to elapse before the value of microscopical investigation was revealed. Mr. E. S. Russell writes, " For Aristotle, as for all anatomists before the days of the microscope, the tissues were not much more than inorganic substances, differing from one another in texture, in hardness and other physical properties. They possessed indeed properties such as contractility, which were not inorganic, but as far as their visible structure was concerned there was little to raise them above the inorganic level. The application of the microscope changed all, for it revealed in the tissues an organic structure as complex in its grade as the gross and visible structure of the whole organism " (" Form and Function," 1916).

In the opening years of last century a

deepening sense of the importance of the very minute happily coincided with great steps in the improvement of the microscope itself. Microscopy entered on a new phase. Profound discoveries in the study of living organisms stimulated a general enthusiasm for the microscope as a fascinating instrument, and further improvements, optical and mechanical, followed. The rapid strides made in biology, with the early history of which the beginnings of microscopy are bound up, led to a desire to penetrate below the surface in other branches of inquiry. The use of the microscope spread to the study of rocks, crystals and metals, and was rapidly followed by valuable results.

Recent years have seen a fresh development. The microscope, now brought to a state of well-nigh perfection, has penetrated into a dozen different industries and its use is rapidly increasing.

What is the microscope but an extension of vision? The early days of microscopy remind us of men learning to use new eyes, turning them here and there with much and varied practice. Then with improved sight and fuller understanding they found themselves able to see beneath the surface of things,

and directed an inquiring gaze on a succession of different interests.

In the following pages we are in effect endeavouring to put before the reader some examples of the use man has made of his enlarged powers of vision and of the benefits he has derived from a deeper gazing into things living and non-living.

CHAPTER III

HEALTH, PUBLIC AND PERSONAL

In 1675, the Dutch microscopist, Antonius à Leeuwenhoek, of Delft, observed "living atoms" in standing rain water, "which had stood but four days in a new earthen pot, glased blew within."

This discovery, made by the simplest of microscopes, he communicated in a famous letter to the then recently incorporated Royal Society of London (*Phil. Trans.*, 1677). It is the first recorded observation of the microscopic animals now called Protozoa.

A dozen years after, the same indefatigable observer showed the existence of a world of still smaller vegetable organisms—the Bacteria.

Partly owing to the inadequacy of the earlier microscopes to resolve the details of these minute creatures—some of which, it should be remembered, are to-day beyond the ken of the most perfect instruments—

it was a long time before their " incalculable powers for good and evil " were appreciated. Not till the nineteenth century was well advanced did Pasteur and Koch, following Pasteur's discovery (1857) of the bacterial causes of fermentation and the detection by Davaine (1854) of the now well-known *Bacillus anthracis* in the blood of sheep, finally establish

FIG. 9.—*Bacillus anthracis*, FORMING CHAINS.

the fact that many of the most deadly diseases known are caused by microbes.

MEANING OF CERTAIN TERMS.—*Microbe* is an abbreviation of " micro-bionta," and is a convenient term applicable to minute living creatures generally, without distinction as to kind, though in popular use it is tainted with disease.

Protozoa are minute animals, nearly all of which consist of a single living cell.

Bacteria, on the other hand, are very minute

plants, some hardly recognisable as cells, and rod-like, globular or thread-like in form.

Bacillus—Bacteria which show a rod-like form are called *bacilli*. The bacilli causing tuberculosis are minute rodlets $\frac{1}{10,000}$ in. in length and $\frac{1}{50,000}$ in. in thickness.

At a somewhat higher level of plant life than the Bacteria are *Moulds*, *Mildews*, and *Yeasts*, included in the group of Plants called *Fungi*. Fungi are plants devoid of green colouring matter (chlorophyll), which live on dead organic matter. Some of them are large, like mushrooms and toadstools; others, like moulds and yeasts, are made up of elements of microscopic size.

In this realm of small things the microscope is indispensable and no small part of the well-being of mankind is bound up with its skilful use. The subject in its entirety is so vast that we must be content with representative examples from the different fields of work.

The malaria microbe is a Protozoon (*Plasmodium*) which enters the blood-stream of sufferers and attacks the red corpuscles. It was first observed in 1880 by Laveran, and immediately focussed the interest of workers in different countries. Their united investigations eventually made it clear that once the

microbe has entered the blood of man, it goes through a well-marked cycle, showing some seven or eight different phases in the blood.

The next step was to prevent the spreading of the disease. How was the parasite conveyed from one human being to another? Sir Patrick Manson, arguing from a wide experience in tropical diseases and from his own observations on the appearances of the microbe in the blood, made the suggestion that some blood-sucking insect was the carrier, probably a mosquito. Following this line of investigation, by long and patient microscopic work Sir Ronald Ross at length fastened the guilt on mosquitoes of the genus *Anopheles*. Step by step, the extraordinary life-history of the microbe was made out. From its entry into the food canal of the mosquito sucking the blood of an infected person, to its final reappearance in the insect's saliva ready to be passed into the blood-stream of the next healthy person bitten, over a dozen different phases may be seen.

Once the cause and the method of infection had been definitely ascertained, it became possible to combat the spread of the disease by attacking the carrier. Drainage of stagnant pools—the haunt of the *Anopheles*

mosquitoes—suffocation of mosquito larvæ by petroleum sprayed on the surface—eradication of the same larvæ by certain small fishes; these measures have given mankind no small control over one of the most deadly scourges of humanity.—And the Panama Canal has been built, too, despite yellow fever.

Of recent years the study of Protozoa has

FIG. 10.—TRYPANOSOME: CAUSING SLEEPING SICKNESS.

assumed such large dimensions that it ranks as a special branch of microscopic science—Protozoology. One of its most important results has been the demonstration of the fact that certain of the Protozoa are closely associated with man or man's domestic animals. Thus the connection of Trypanosomes and sleeping sickness in man and tsetse-fly disease in cattle, Glugea and silkworm disease, Treponema and syphilis, Babesia and Texas fever in cattle, Amœbæ

and intestinal inflammation associated with dysentery. Directly comparable with Protozoa are the phagocytes or wandering amœboid cells normally present in the human body, whose beneficent activities in combating intruding protozoa and bacteria were first shown by Metchnikoff.

The microscopy of Protozoa and Bacteria

FIG. 11.—SPIROCHÆTES: CAUSING RELAPSING FEVER.
B. Red Blood Cell.

calls for the most skilful use of the best instruments and the most refined techniques. Before going on, however, to discuss technicalities, let us say something about the activities of Bacteria. It should be clearly understood that only some bacteria are harmful to man. Many are useful, as for example, in cheese-making, brewing and leather curing; many are normal inhabitants of the intestine of healthy people and only

become dangerous when they get out of hand. It must not be forgotten that though it is relatively recently that the microscope has made known their activities, bacteria have from time immemorial played an indispensable part in the economy of nature. Their food is organic matter, mostly proteids, and "putrefaction" is the breaking down of proteids by ferments elaborated by bacteria. Eventually the dead animal or vegetable matter is reduced to a soluble condition, in which it can be absorbed, not only by the bacteria themselves, but by green plants which, under the action of light, build it anew into proteids.

As Sir Ray Lankester has pointed out, "Were it possible to remove from existence all bacteria, the earth's surface would be encumbered by the highly elaborated proteids forming the dead bodies of animals and green plants, and the organic elements would be locked up in them." These proteids "would remain undecomposed though dead, and the chain of life would be broken."

The bacteria in the soil are the basis on which the whole of agriculture rests. They are responsible for the nitrates which are a necessity to the growing plant. Some of them

—called "nitrogen-fixing" bacteria—can actually capture free nitrogen from the atmosphere. Their numbers are incalculable. Of large visible creatures a count of an acre of wheatfield at Rothamsted showed $2\frac{1}{2}$ million insects, half a million earthworms, three-quarters of a million millipedes and centipedes and a quarter of a million others. But when we learn that "in a teaspoonful of good arable soil there are more living organisms than there are men, women and children in the United Kingdom," we have some idea of the pigmy population of the soil.

The fact that the majority of infectious diseases in man and animals (and some of those in plants) are caused by harmful bacteria has resulted during the last half-century in the development to its present dimensions of the science of Bacteriology. The intruding microbes may enter the system in many ways—by the mouth, the nose, wounds in the skin, the bite of fleas and lice, etc., and may multiply in such numbers that natural safeguards, such as phagocytes, cannot cope with them. They secrete powerful poisons which are often speedily fatal to the body tissues. The tubercle bacillus, discovered by Koch in 1882, the *Bacillus typhosus* of

typhoid fever, the Klebs-Löffler bacillus of diphtheria (1883), the *Bacillus tetani* of lockjaw, the "comma" bacillus of cholera, the *Bacillus mallei* causing glanders in horses, the *Bacillus anthracis* of anthrax are typical examples of pathogenic bacteria. A vivid account of the ravages of diphtheria and tuberculosis will be found in a cognate volume in this Library (No. 17, "Health and Disease"), where the whole question of man's well-being is dealt with.

There are several lines of investigation to be followed up in studying microbes :—

(*a*) A stained film of the blood or other fluid containing them, or a "hanging drop" preparation may be examined with the microscope, or a thin section of infected tissue examined.

(*b*) Some protozoa and most bacteria can be grown or "cultured" in various media (gelatine, broth, agar, blood-serum, etc.). The general characters of the growth are noted and the number and characteristics of the "colonies" observed under a low-power objective. The bacteria may be *aërobic*, that is living in air; or *anaërobic*—thriving best without it.

(*c*) The possible pathogenic properties may be tested by inoculating with a pure culture of the microbe guinea-pigs or other laboratory animals. The symptoms of the animal are noted and its tissues are afterwards examined microscopically.

Microscopic Practice.—It will thus be seen that the microscope is a *sine qua non*, and owing to the minuteness of the organisms, a combination of eyepiece and objective totalling a very high magnification—1000 diameters and over—is essential. This calls for particularly skilled use of the sub-stage condenser, for, as was pointed out in Chapter I, accurate adjustment of the illumination falling on the object to be examined is the key to good microscopy. It is all too easy to observe "diffraction" images. The stained film is placed on the stage, the illumination adjusted provisionally with the medium-power objective and the highest power—the "oil immersion" objective of $\frac{1}{12}$ in. focus—revolved into position. It may be said in passing that some of these micro-organisms are very difficult to stain, especially "cysts" and "spore" forms. "Acid fast" bacilli in particular require a powerful stain containing a mor-

dant, usually aided by the application of heat.

A drop of cedar oil is now placed on the film and the objective lowered into it, focussed and kept immersed in it. The final adjustment of the sub-stage condenser is then made to ensure a beam of light appropriate to the objective in use.

Most lenses of very high power are constructed as " oil immersion " lenses and cannot be used " dry." Cedar oil has nearly the same refractive index as glass, and the filling of the space between lens and slide with a liquid which is practically equivalent to glass prevents refraction of light rays from the surface of the slide out into the air. Instead, these rays find their way through the front of the objective. A greater amount of light from every part of the preparation under examination thus enters the microscope, resulting in an increase of resolution.

Care in adjusting and co-ordinating every part of the microscope and its accessories when working at high powers approaching the limits of microscopic vision is of paramount importance.

In the case of a " hanging drop " preparation of bacteria, the observer will probably

study it under "dark ground illumination." A small drop of the fluid containing the microbes is placed on a coverslip and inverted over a hollowed slide. This is then placed on the microscope stage and the organisms examined alive, note being taken especially of their powers of movement and the rate at which they multiply. Under dark ground illumination they appear brilliantly illuminated against a velvety-black background. It may be said shortly that this is effected by sub-stage arrangements which prevent any light coming *through* the objects examined, but which permit light to be shed on them *obliquely* from all sides, so that the only light which passes up the microscope tube is that reflected by the organisms themselves, which consequently appear bright on a dark ground. Modern methods of dark ground illumination must be reckoned among the most valuable developments in microscopy (see Fig. 12).

The part played by such investigations behind the scenes of our ordinary everyday life is not so well known as it should be. One of the conditions of our existence is water. Every human being needs water. A considerable proportion of our bodily tissues consists of it. As Sir Arthur Shipley has

reminded us, "Even the Archbishop of Canterbury comprises 59 per cent. of water" ("Life," 1923).

As water is the thriving ground *par excellence* of microbes of all sorts, means must be

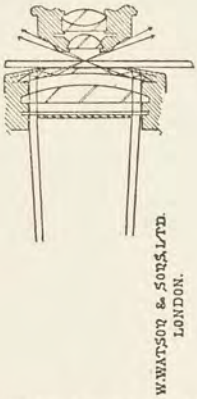


FIG. 12.—DARK GROUND ILLUMINATOR.

taken to make certain that water for man's use is free from those kinds which are injurious or undesirable. Control follows knowledge and the lessons learnt in the laboratory are put to practical account by those whose business it is to ensure a pure water supply to our towns. A magnification of 50 times

will show a host of small animals and plants in ordinary river water especially in spring. Many more flourish in reservoirs and occasionally in such numbers as to throw the purifying filters out of action. In 1913, an overgrowth of the minute vegetable organism *tabellaria* gave rise to a geranium-like taste and smell in the water supply of a part of London. Similarly a " cucumber-like " taste in New York water in 1921 was traced to a growth of *synura.*

A high-power examination of unfiltered water will disclose countless bacteria, comparatively few of which are harmful unless in excessive quantity. Thus water containing 20 per cubic centimetre of non-virulent bacteria might reasonably be considered safe to drink. A proportion of 2,000 per cubic centimetre would undoubtedly result in such a supply being condemned. Virulent bacteria, such as the deadly typhoid bacillus, are another matter. One of the commonest bacteria found in water is the *Bacillus coli,* a usual inhabitant of the intestine of man and various animals. The detection of this bacillus in large numbers usually points to fæcal contamination. The *Bacillus coli* may under certain conditions be pathogenic, but

the point is that the typhoid bacillus, which is difficult to detect (and incidentally very like *B. coli* in many respects) is also carried in fæcal matter, and where the one is the other may be.

It is relieving to know that over 98 per cent. of the bacteria and practically all other micro-organisms are kept back by modern filtration processes. Thus in 1902, the Massachusetts Board of Health found that continuous filtration through 45 inches of sand removed 99·9 per cent. of the typhoid bacilli, 99·8 per cent. of the *Bacillus coli* and 98·7 per cent. of the total bacteria present in the water. (34th Report, 1903.) The work of the filter must, however, be checked and controlled week in, week out, by careful examination of the water.

Chemical examination is, of course, a necessary adjunct to bacteriological examination. But only the bacteriologist can ascertain the quantity and the nature of microbes present. With microscope and culture plate, he will determine the number of bacteria present per c.c. of a given sample, what kinds of bacteria are present, and if, as is usually the case, he is on the look out for any particular microbes he will ascertain in what

proportions they occur. (See " Bacteriology," in this series.)

The microscopic investigation of the air we breathe might at first sight seem to be of equal importance to that of water, but this is not quite the case. In the case of bacteria it must be remembered that these naturally require a moist environment and only a few survive prolonged drying: then the air is vast in amount, there is plenty of room. The result is that when a measured quantity of air is examined, harmful microbes are rare, unless the sample of air was taken in the immediate vicinity of a source of infection, such as a person suffering from influenza, where coughing may distribute minute droplets of moisture containing the bacilli. On the other hand, the bacilli of tubercle, diphtheria and anthrax, which can resist drying, can be blown about and still retain their vitality should they chance to light upon a suitable nidus. The examination of the micro-organisms in the air invariably shows a greater or lesser number of harmless bacteria, either floating free or caused by dust, and numbers of minute fungi, chiefly moulds and yeasts. The spores or reproductive elements of these are almost always present, especially

the common blue-green mould. A cubic metre of air from the suburbs of Paris in summer time contained 170 bacteria and 145 moulds. In the interior of the city the results were 9,845 bacteria and 2,500 moulds.

It is interesting to learn that, after a careful

FIG. 13.—DUST PARTICLES IN THE AIR; INSIDE A WORK-ROOM.

investigation of the air in the Houses of Parliament, it was found that the air in the debating chamber proved remarkably pure from a bacteriological point of view, that it contained no microbes harmful to man and only a few harmful to animals. Pollution of the air by smoke and soot, however, is a serious matter in our big industrial centres. The particles in

a measured volume of air may be examined microscopically, after being collected on a microscope coverglass by means of an ingenious instrument designed by Dr. J. S. Owens (*Proc. Roy. Soc.*, 1922). The coverglass, which shows a fine ribbon of deposited dust, is mounted dry on a slide and the number and nature of the constituent particles are ascertained. The counting is done with a square-ruled micrometer eyepiece and an "oil immersion" objective.

Dr. Owens, in a recent paper, has pointed out that the air over the North Sea off Spurn Head contains 140 particles per cubic centimetre, while the atmosphere of London has 4,000–5,000, rising during a fog to 100,000 per c.c. Further, in London, there is a daily fluctuation; the particles are fewest in the early morning, increase till a little past noon, and then decrease again. They are least in amount on Saturdays; and are derived very largely, *not* from factory smoke, but from household chimneys.

Surely one of the most interesting methods in microscopy is Dr. Owens' way of finding out the electrical state of suspended dust. He uses a shallow cell of thin vulcanite with a glass floor and roof, in which lie two copper plate

electrodes whose terminals are outside the cell. "This could be placed on the stage of the microscope, the two electrodes charged, and smoke particles, drawn into the cell through holes provided for the purpose, could be observed between the electrodes. The cell, when illuminated by dark ground illumination and examined with a $\frac{2}{3}$ in. or 1 in. objective with a $\times$ 10 or $\times$ 20 eyepiece, enabled the individual smoke particles to be seen suspended in the air, and, when the electrodes were charged, some moved towards one and some towards the other, and thus the nature of the charge could be ascertained."

It is well known that many of the commoner foodstuffs may harbour harmful micro-organisms. Of these the most important are milk (see "Health and Disease") and meat, especially preserved or tinned meat. The peculiarly deadly form of "meat poisoning" known as botulism is produced by the *Bacillus botulinus*, found by Van Ermengem in 1896. It is a large bacterium from one to two five-thousandths of an inch in length and produces an extremely potent toxin—one-thousandth of a cubic centimetre of which will kill a guinea-pig.

Meat, butter, cheese, "shell-fish" like

oysters and mussels, which may have come from fouled waters, bread that may be manufactured under unclean conditions, all these come under the eye of the public health officers, who by constant checking ensure the purity of supplies intended for human consumption. The question of the handling of unwrapped bread is one in which there has been a growing interest of recent years, for in Great Britain it has been the exception rather than the rule for bread to be marketed in sealed coverings. According to Howell (1912), a hundred loaves were taken from different shops of divers degrees of cleanliness in Chicago. Some were wrapped (that is, sold in sealed coverings), and some were unwrapped. The results were—

	Under 1,000 bacteria.	1–10,000.	Over 10,000.
Wrapped	85%	15%	0%
Unwrapped	38	45	17

In over thirty instances harmful kinds were found.

On another occasion, three loaves sold in New York as free from human handling after baking showed 600, 200 and 280 bacteria respectively. Three similar loaves were then taken, given to three different groups of persons to handle, and examined in the same way as

the first three. The numbers of bacteria found in the handled loaves was 15,140, 1,080, 1,360. It is but fair to add that on this particular occasion none of the bacteria were of a definitely pathogenic character, but the risk is obvious. Another lot of similar loaves purchased unwrapped had from 2,720 to 325,500 bacteria each, and four out of the six showed the presence of *Bacillus coli*.

Ordinary "musty" bread is due to the presence, not of bacteria, but of a mould (*Mucor mucedo*), one of the lesser fungi. It thrives in damp flour, and though baking kills it, the mustiness persists in the baked loaf.

There is another microbe which, though not common, is interesting in that its presence gives to bread a red, blotched appearance. It is said that this infection was observed during the siege of Troy and that the Roman soldiers' rations were on occasion tainted by it. Perhaps Seneca examined it "through a glass globe filled with water." Various records exist of its appearance in England during the Middle Ages, and an epidemic of "bloody" bread occurred in North Italy during the earlier part of last century.

MEDICINE.—It is common knowledge in a general way that the microscope is a useful

instrument in the practice of medicine. It would be difficult, however, to obtain a more striking testimony than that afforded by "The Link between the Practitioner and the Laboratory" (Fletcher and McLean, 1920). The authors enumerate over a hundred and fifty diseases in which laboratory tests of various kinds are necessary or advisable in order to assist diagnosis, treatment or prognosis of disease. Perusal shows that in no less than 80 per cent. of these diseases, microscopical examination of some kind is required in one or other of the tests. A bacteriological examination is needed in 40 per cent. of the cases, an examination of the blood in 32 per cent., microscopical examination of urine 14 per cent., of fæces 10 per cent., of the minute structure of bodily tissues 12 per cent., and there are other cases accounting for 10 per cent. more.

"In order to obtain this useful knowledge it will be necessary to examine the human blood and other juices, frequently, with the *Microscope*, in every Condition, and under every Distemper, as well as in a State of Health; by which we shall have Ocular Demonstration of its different Appearances in each State, and of the Changes it undergoes; and by Experiments of various Mixtures with it, may possibly

discover by what means it can be altered from one Condition to another. . . . Would our learned Physicians who are best able to judge of such Matters, be induced to take this Method into their Practice, it is reasonable to believe, that in a few Years the Causes of Diseases would be better known, and the Art of Healing brought to a much greater Certainty than it is at present." Thus wrote Henry Baker in 1742 : surely a prophecy !

The modern pathologist in examining microscopically the blood of a patient has two main objects in view. 1. To ascertain the existence of parasites (microbes of disease). 2. To ascertain the number and certain proportionate relations of the corpuscles (red cells and white cells) of the blood. Either or both of these may be valuable according to the case under consideration.

It has been said by a well-known authority that " a well-prepared blood-film is a page of information to him who can read it." An extremely thin film of blood is spread out on a slide, appropriately stained, and examined with a high power. The various blood corpuscles are fully differentiated by the colouring matters of the stain and any parasites or other foreign bodies can be observed. The

number of corpuscles or cells in the blood is enormous. The blood of a healthy man contains over five million red cells per cubic millimetre. There may be another million or more if he is a dweller in high altitudes.

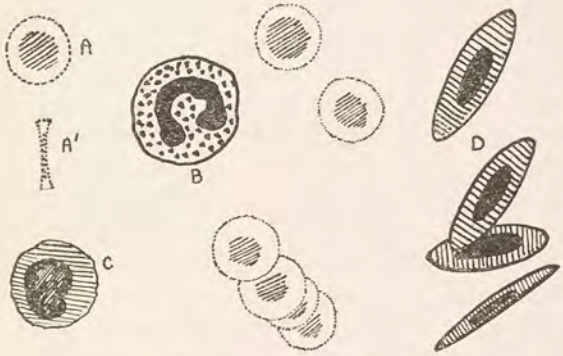


Fig. 14.—Blood Cells.

A. Human red blood-cell. A'. The same in side view. B, C. Human white blood-cells. D. Red blood-cells (nucleated) of a fowl.

White cells are much fewer; numbering round about eight thousand per cu. mm., though this number may be very greatly increased in certain illnesses. The principle made use of in determining such large numbers is to dilute a small and very accurately measured volume

of blood, either 100 or 200 times in the case of red cells. White cells being more manageable in numbers only need a 10 times dilution. A drop of the dilute mixture is then placed on a special microscope slide, so constructed that when the coverglass is laid on, the diluted blood forms a layer exactly $\frac{1}{10}$ mm. thick. The slide is ruled in squares of known dimensions, usually $\frac{1}{10}$ mm. square. The red cells (or white cells) within one square counted under the microscope (it is usual to take the average of a number of squares) would be the number for $\frac{1}{1000}$ cubic mm. This multiplied by the dilution and then by 100 would give the number of cells per cubic mm. of blood.

To the minute investigation of the bodily tissues we shall have occasion to refer later. But we should notice that the results of such examinations are frequently of much value in diagnosis and often contribute greatly towards ascertaining cause of death. Sometimes they have a weighty bearing in courts of law, particularly in capital cases. Needless to say on such occasions most skilful microscopy and a well-balanced judgment are necessary.

Crime.—Those whose duty it is to investigate crime find the microscope an indispensable tool.

While they do not aspire to emulate the feats of Sherlock Holmes in regard to tobacco ash, there is no end to the variety of their investigations. It is often of the highest importance to ascertain the origin of blood splashes. The red blood-cells of all mammals save the camels are circular in shape and devoid of a "nucleus," but those of birds, reptiles and fishes are definitely oval and nucleated. A blood-stained knife produced in a murder trial was said by the accused to be the result of cleaning fish. Microscopical examination showed the stains to be those of mammalian blood, and further elaborate tests showed that they were human. In another case, the defendant was accused of stealing fowls. He alleged that certain blood-stains on his clothes had been caused by the blood of a rabbit. But the corpuscles were oval and nucleated, and a conviction ensued. The presence of blood and sometimes its origin may be often detected by treatment to show "blood crystals." The red colouring matter of the cells—hæmoglobin—may be dissolved out, treated with reagents, and, upon evaporation, forms characteristic crystals, rhombic in shape.

Stains and blood marks on linen or other cloth are examined, not only in respect of the

stain, but also in regard to the material itself. The nature of the threads or fibres, the texture, slight differences in the weaving, and so on, may render it possible to determine how, when, and where the cloth was woven. Similar information may be gleaned in regard to paper documents, important in the case of forgeries or spurious notes. Ropes, brushes, hats, woollen stuffs, all demand an examination of their constituent fibres and hairs. Hair often yields valuable information. Different animals show characteristic differences in the hair which clothes them, especially in the inner axis or core; and animal hairs differ from human hair. Even the hairs of different races of mankind show characteristic differences. Examination of the roots will often disclose whether the hair has dropped out naturally or has been forcibly pulled out.

An interesting case happened some years ago, when a native servant in Northern India was murdered during the night. Suspicion naturally fell on his fellow-servants, who were submitted to examination. On the clothing of one of them, one or two fresh blood-stains were found. These at once showed the presence of round mammalian blood-cells. This was only a modest clue, but further

microscopic examination revealed in addition the presence of numbers of young stages of the minute parasitic worm Filaria of a type peculiar to man, whose presence in the blood causes a well-known tropical disease. Investigation showed similar bodies in blood from the deceased person. It proved a very strong point against the accused person, who was found guilty of the crime and sentenced accordingly.

CHAPTER IV

THE CRUST OF THE EARTH

From the very earliest times man has been interested in his earth. Among the earliest natural objects he contemplated were the mountains and valleys, the rivers and the rocks; and his primitive imagination played round these and the clouds above, as the myths of all nations abundantly testify.

From very early times, too, man has found use for the products of the earth. His first weapons and implements were stones. These gave place to roughly dressed stones, shaped and polished stones, and these, as his earth knowledge grew and he learnt that metals were to be gleaned from the rocks, to instruments of copper, bronze and iron. The founder of petrology and the first prospector both emerged with primitive man.

The pursuit of the modern science of Geology—earth study on a strictly scientific footing—is of relatively recent date and only

came into existence towards the close of the eighteenth century. But even with such a comparatively short life it has already clarified our ideas and enlarged enormously our knowledge of the fabric of the earth, with steadily increasing results of an economic nature. The province of Geology is a wide one. Included in it are the study of the materials of which our earth is made and their arrangement in space and in time : the investigation of the movements and changes below and above the surface which have resulted in the physical configuration of the globe as we know it, the study of the periods of the earth's history and the procession of life on the earth.

In a vast subject, the investigation of the nature of the different rocks which go to the building of the earth's crust stands out as of fundamental importance to the geologist. In this branch of geological study, the microscope has made possible contributions to knowledge, of a kind otherwise almost unobtainable, which have had far-reaching practical effects.

The rocks which compose the earth's crust have originated in three different ways. Some are *igneous* rocks like lavas, granites and porphyries, formed under great pressure and amid great heat under the surface of the earth.

Others, like clays and slates, sandstones and conglomerates, are *sedimentary* rocks formed at the surface by the action of wind and water. In a third category are *metamorphic* rocks, quartzites, schists and gneisses, which are derivatives from the other two types.

In the early days of geology, the nature of rocks was not well understood.

Werner of Freiburg (1749–1817), who seems to have been the first to distinguish geological formations or rock masses from their constituent rocks, attempted to arrange various rocks according to their mineral composition. And for a time mineral composition was the chief basis of classification, in so far as the mineral composition of a piece of rock can be judged in the hand with the naked eye or simple lens. Others, like Von Leonhard and Brongniart took into account the general structure or texture, and supposed origin. Chemists attacked the problem, with some success. But chemical analyses of rocks, though they have proved of great value, are not sufficient for geological needs. Knowledge of the physical structure of rocks, of what minerals they are made up and how these minerals are distributed, is essential, but this knowledge cannot be gleaned from the study

" of superficial characteristics and approximate chemical analyses."

" By 1850," writes Professor H. E. Gregory, " the possibility of increase of knowledge through the study of rocks appeared to have been exhausted; no further steps of advance seemed possible, for the components of fine-grained rocks, lavas, and schists were beyond the reach of observation, and there appeared to be no satisfactory means of distinguishing the varieties of feldspars, the most abundant ingredients in the commonest rocks. Petrography had come to a blank wall."

Then a new era dawned. In 1858, Sorby showed that the microscope could be applied to rock investigations in a way that would yield results of first-rate importance and of the highest value in geological research. A thin piece of the desired rock could be ground down sufficiently thin for it to be placed on the microscope stage and examined like any other transparent object. In polarised light this " brings to view and differentiates minerals even in apparently homogeneous rocks."

The result of this was that geologists all over the world plunged into the microscopic study of rock-structure. The various rock-

forming minerals were identified, and their physical and optical properties became known. When the nineteenth century closed thousands of rocks had been studied and classified and their minute internal structure described. An enormous amount of valuable data has thus been accumulated, which, together with the employment of improved chemical methods, and the more recent application of physical chemistry, has allowed the study of rocks to become the science of the rocks—Petrology.

What, then, is the exact nature of the information yielded by the microscope about rocks and minerals, and how is it obtained?

To begin with, we must understand how such intractable objects as rock pieces are prepared for microscopical examination.

To William Nicol, of Edinburgh (1768–1851), physicist and lover of crystals, microscopists owe the method of preparing sections of hard material. As far back as 1831 Henry Witham, of Lartington, published "Observations on Fossil Vegetables"—"as seen through the Microscope,"—and takes pleasure in laying before his readers a full account of the process, for which he is indebted to Mr. Nicol. The process he describes in minute detail is substantially the same as is to-day universally

used for rock sections, to which it was first applied by Sorby.

A thin chip of rock, or a machine-cut slice the thickness of a coin, has one side ground smooth by hand or by machinery, an abrasive (emery or carborundum) being employed. After polishing, this side of the specimen is firmly cemented to a piece of plate glass or a microscope slide by means of Canada balsam. The other side is then ground down till the rock slice is of the desired thickness—about $\frac{1}{1000}$ in. thick. If the specimen is mounted on a microscope slide, nothing remains but to clean it, drop on a little balsam and add a coverslip; if on plate glass, it has to be melted off and transferred for mounting to a microscope slide. By this means a section of a dense black rock can be produced so thin as to be almost invisible and transparent enough to see fine print through.

The geologist's microscope—usually called the petrological microscope—differs in several ways from the ordinary instrument. In addition to various observations in ordinary light, a further class of observations in "polarised" light has to be provided for. This entails certain accessories not commonly found on other microscopes, the chief of which are a

polariser below the stage and an analyser placed in the tube above the objective, together with a special eyepiece with cross hairs : the stage is usually a rotating stage with a graduated scale of degrees round the edge.

Polarised Light.—The term polarised light needs explanation. Polarised light is the name given to light rays which have been physically altered so that they travel along

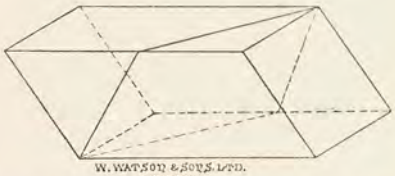


Fig. 15.—Structure of a Nicol Prism.

vibrating in *one definite direction* at right angles to their path. Ordinary (non-polarised) light vibrates in *all directions* at right angles to its path.

Such polarised light may be produced in several ways, but in the petrological microscope the light is polarised by refraction on its way through the polariser situated beneath the stage of the microscope. The polariser and the analyser each consist of a transparent

cleavage piece of the mineral calcite (Iceland spar) arranged to form a "Nicol prism." Long ago Bartholin (1669) observed double refraction in crystals of Iceland spar. Refraction, it will be remembered, is the bending of light on passing from one medium into another, exemplified by the bent appearance of a spoon in a glass half filled with water. When a ray of light is not only bent, but split in two, double refraction occurs.

The two bent rays thus emerging from a piece of Iceland spar are both polarised, and Nicol (of section-grinding fame) devised, in 1828, a method of eliminating one of these rays and allowing the other to pass. His method is embodied in the nicol prism. When the two nicol prisms or—more shortly—the two "nicols," polariser and analyser, of the petrological microscope, are in certain definite positions relative to each other (are parallel), the beam from the polariser can pass, unless otherwise checked, straight up the microscope tube through the analyser to the observer's eye. The field of the microscope accordingly appears illuminated. If, however, the nicols be "crossed," the beam is checked by the analyser and the field is consequently dark. The nicols remaining crossed a slip of

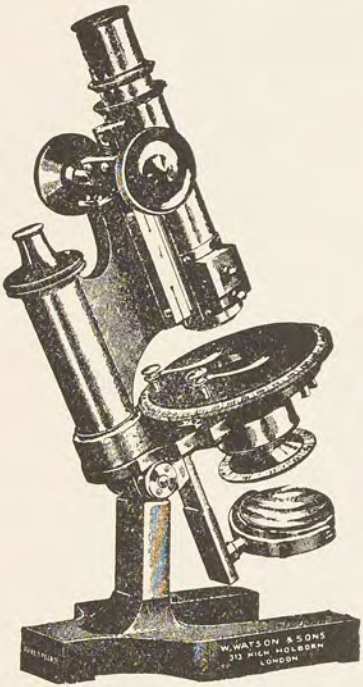


FIG. 16.—THE PETROLOGIST'S MICROSCOPE.

(The objectives and nosepiece are not shown. The analyser is mounted at the lower end of the tube. The polariser below the stage.)

glass or other simple-refracting substance may be placed on the microscope stage. No change will be ordinarily observed : the field remains dark. But if this be replaced by a substance which exhibits *double refraction*, the beam from the lower nicol—the polariser—is thereby modified in such a way that the field is more or less bright. In a rock section, therefore, we can at once distinguish any glassy constituents present by the fact that with crossed nicols they show up as dark patches against the rest of the field : and the examination in this way of a section whose ground mass proved to be glass would enable us to state immediately the origin of the rock as volcanic.

Rock Structure.—Whatever their differences in origin all rocks are aggregates of minerals: quartz, felspar, calcite, zircon, hornblende, magnetite, orthoclase, to name a few at random. Minerals are the units of rock structure. Like nearly all inorganic solid matter they assume definite crystalline forms, and it is of interest to note that rock microscopy depends in no small degree on the fundamental fact that crystalline form is the outward expression of chemical constitution. Hence by ascertaining the physical properties of a mineral crystal we can identify it—say of what substance it is composed—

without recourse to chemical methods. For this purpose, the microscope becomes in fact a crystallographic optical instrument. The

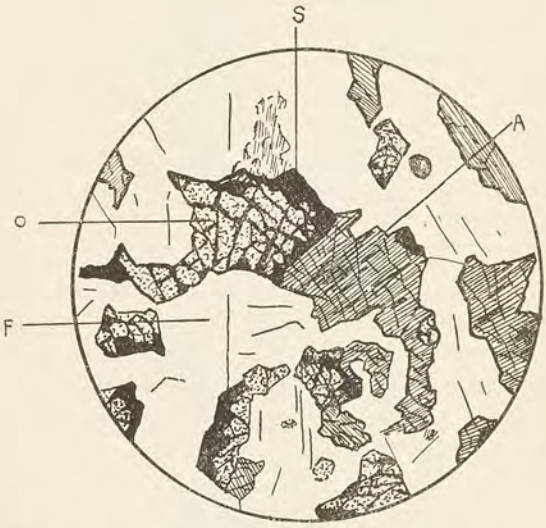


FIG. 17.—ROCK SECTION: *olivine-gabbro, Banffshire.*
A. Augite. S. Serpentine. O. Olivine. F. Felspar.

matter is complicated by the fact that whole crystals rarely appear in sections, and observations have to be made as a rule from numbers of fragments.

Mineral Crystals in Polarised Light.—Crystals show a great variety of forms, which are classified by crystallographers into six groups called crystal systems. Rapid identification of a mineral may often be made by recognising the crystal form.

All crystals, save those of the cubic (or regular) system, together with certain exceptions among the other systems, exhibit double refraction, and hence appear bright or coloured between crossed nicols; but by making use of the graduated rotatory stage a certain position can be found when the specimen appears dark. This is the position of extinction for the particular crystal, and is valuable in referring a crystal to its system. Further, the measurement of extinction angles will sometimes serve to differentiate two minerals present together in one section, hornblende and augite, for example.

Various colour appearances yield important information. Doubly refracting mineral crystals between crossed nicols show characteristic interference tints. With the analyser removed, a further set of phenomena may or may not be observed when the polariser is rotated, and the pleochroism of the mineral in question noted.

Again, some structural peculiarities imperfectly seen under other conditions may be shown up. Thus polarised light will often reveal " twinning " in mineral crystals which show no sign of it in their external form.

In all these observations low-power objectives are needed, and a sub-stage condenser is not required. By the use of a sub-stage condenser, which converges the beam of polarised light on the specimen, further information may be gained. The uniaxial or biaxial nature of a doubly-refracting crystal —an important point—can be determined by examining the interference figures and the optic angle of biaxial crystals measured.

It will be seen, therefore, how much microscopic examination in polarised light tells regarding the characteristic physical properties of crystals. It is thus invaluable in identifying or determining the particular minerals present, in distinguishing one mineral from another, and so elucidating the nature of the rock under examination. Von Kobell has poetically described the enthusiasm of the early investigators as the relations between the physical and chemical characters of minerals became known. " The development of the optical relationships opened a magnificent

field of wonderful phenomena which can be described as a garden gay with flowers of light, charming in themselves and interesting in their relations to the forces which guide and govern the regular structure of matter" (quoted by Ford, 1918).

Observations in Ordinary Light.—It must not be forgotten, however, that a very great deal of information is obtained from the inspection of a rock section in ordinary non-polarised light. The analyser and the polariser are moved out of the way and the light from the mirror passes unaltered. Examination then shows the general texture of the rock: the forms of the constituent minerals, thus indicating the order in which they crystallised; whether any minerals are present which possess characteristic coloration; whether any alterations are in progress of any of the minerals (such as the kaolinisation of felspar); as well as cleavages, cracks and inclusions of one sort or another. The size of particular constituents may be measured and the relative proportions of different ingredients accurately estimated. Each bit of information counts in determining the exact nature of the rock.

When a piece of quartz is mounted in

Canada balsam and examined with a low-power objective in ordinary light its edges will appear indistinct and the piece itself will not be well seen. On the other hand, fragments of garnet and corundum (of which the ruby and the sapphire are varieties) are clearly outlined and stand out strongly in relief. The reason is that the refractive index of quartz (1·55) is almost the same as that of Canada balsam, while the indices of garnet (1·81) and corundum (1·76) are much higher. This principle can be employed to determine the relative refractive indices of two closely adjacent minerals in a rock section. In this case, on lowering the objective slightly a band of bright light will appear to pass inside the less refractive substance.

Rock Study.—To sum up, the microscopical study of rocks yields exact knowledge of their composition as determined by the nature and proportions of their mineral constituents. It makes clear the meaning of the texture, or appearance apparent to the naked eye, by showing the size, the shape and the arrangement or disposition of these constituents. By deduction from these facts we obtain knowledge of the formation and growth of minerals, of the physical conditions under

which the rocks have formed, of the changes —physical and chemical—which the rocks have undergone since their formation, and of the relationships which rocks bear to each other. Without the microscope determination of the principal constituents of a rock is an elaborate and lengthy business. Determination of accessory constituents is even more difficult. Very little can be said about the texture of igneous and metamorphic rocks without recourse to microscopic examination. Knowledge regarding rock relationships can hardly be obtained, otherwise than through the microscope.

We see then that the application of the microscope to the investigation of the earth's crust, which placed the geologist on the same footing as the zoologist and botanist in regard to the minute study of his subject, has consolidated firmly the foundations of petrology. Petrology is one of half a dozen sub-sciences among which the vast subject-matter of Geology is divided. These must not be thought of as watertight compartments, but rather as partner sciences all contributory to the main purpose—the study of the structure and history of the earth. The microscopical gleanings of petrology therefore aid other

partners, and they in return illumine the findings of petrology and suggest new lines of investigation. To Dynamical Geology, petrology yields microscopical evidence of the agencies that have been concerned in the formation or alteration of rocks; to Structural Geology it gives evidence that the arrangement of rocks as seen in mass in the earth's crust may have its counterpart in the minute structure of the rocks themselves, and enables distinctions to be made between rocks which may be associated in the field and apparently similar, but which are in reality unrelated to each other. Palæontology—the study of fossils—is informed that some rocks, *e.g.*, certain limestones, are composed of microscopic organisms; while Stratigraphy, the history of the earth, learns that the rocks contain microscopic evidence which helps the description of past conditions, both organic and inorganic, of the globe.

This, then, is the part played by the microscope in geology—the science of the earth. And since the value of sound geological knowledge as the basis of modern exploration of the earth's resources has become patent the methods as well as the fruits of geology are being more and more utilised in the business

of extracting and transforming the mineral products of the globe. As in other branches of human activity, the microscope, from being the tool of the scientist pure and simple has become a well-nigh indispensable adjunct to the practical man; sometimes as a means of control in whole or in part, sometimes pointing the way to refinements of manufacture, frequently as a critical test of workmanship, but always valuable.

Let us think for a moment of the extent to which mankind makes use of the mineral products of the earth's crust. This is the age of aluminium and aluminium is probably the most abundant metal in nature. It is a constituent of between twenty and thirty minerals, of which bauxite is perhaps the chief. Iron, gold, silver, copper, lead, platinum and other metals; coal; oil; sandstone, granite and limestone for building materials are familiar to everybody. Then there are nitrates, phosphorus and potassium for agricultural fertilisers; mercury for high explosives and calomel powders; bismuth for metal alloys and *X*-ray meals. Magnesium, as well as being the origin of nursery medicines, is likely to become of great importance in the development of aviation, as the basis of extra-light

metal alloys. Uranium-containing minerals yield radium; fluorite goes to the making of the finest apochromatic lenses of the microscope. Chromium, vanadium, tungsten, cobalt, and manganese render possible the various steels used for armour plating, projectiles and high-speed tools. Carbon crystallises in one way into diamonds for the few and in another forms graphite for the lead pencils of the many. With the exception of a few of organic origin such as coral or pearls, and of the synthetic gems made in the laboratory, precious stones are crystals of minerals, dislodged from the rocks and valued for their splendour of shape and colour, their hardness and rarity.

One of the most remarkable developments of recent times has been the introduction of petroleum as a source of energy. Year by year sees the use of oil fuels steadily extending, and thus further discoveries of oil wells on the one hand, and their judicious conservation on the other, are at present problems of world importance. Oil is widely disseminated through the upper part of the earth's crust, especially in those layers of rock which have been accumulated at the surface, as distinct from igneous rocks upheaved from under-

neath. The oil is in all probability mainly derived from the entombed remains of once living animals and plants, profoundly changed under various conditions of temperature and pressure. Thus slate, which is a rock formed from consolidated mud, into which the dead bodies of animals easily sank, is often saturated with oil, though in a form which does not permit of easy refining. Most of the world's oil supply comes from sandstone rocks, in which the oil has collected. Sandstone is composed of grains of quartz more or less closely packed or cemented together. Between the grains the oil accumulates. The smaller and more angular the grains, the less room for oil; the larger and rounder, the greater the available space. Consequently sandstones of great porosity are very favourable to rich oil deposits, especially as migration of the oil tends to take place under pressure from less porous rocks into the more open sandstone. Thus in slate the lighter oil will percolate through and the heavier residue will be left.

When oil-bearing sandstone is hedged about by layers of impervious rock, the oil will be trapped—will be unable to drain away—and a reservoir of oil will result. The establishing

of an oil well means the tapping of this reservoir.

It follows, then, that a thorough knowledge of earth structure is a necessary starting point when oil drilling is in contemplation. It is the business of the geologist to examine a likely region and ascertain that it contains formations of the right kind and the disposition and shape of the rock layers. Once upon a time the various strata of oil-bearing rock were laid down on the level, but subsequent earth movements have tilted here and displaced there till a single layer followed out may have a series of ups and downs like a barometric record.

A probable site having been chosen, drilling begins. As the drill carves down through layer after layer, sample after sample is taken *en route* of the shattered *débris* ground out by the cutting bit. These samples are handed over to the geologist, who examines them to ascertain definitely what rocks are being cut through. He will separate out with bromoform the heavier mineral constituents and the microscopic study of them will show the assemblage of minerals present. He will also study the samples for traces of organic remains. As the material is in a finely-com-

minuted condition microscopic study is essential, and the aim of it is to determine the nature of the rock, and so be in a position to judge of the likelihood or otherwise of oil having collected in the vicinity. A series of samples which proved to be from rocks of a thoroughly unfavourable nature would enable a probably fruitless boring to be discontinued and time and money would be saved.

When sandstone is reached the porosity of it is determined. The size and shape of the grains are clearly shown by the microscope. The actual amount of pore space can be learned by saturating a known amount of dry rock with water. Ten per cent. of pore space might indicate a possible oil yield of 776 barrels per acre-foot. In addition to the pore space, the degree of moisture of the sandstone has to be taken into account. It is not likely that oil will be found below water, as its lower specific gravity would tend to bring it to the top. Hence the finding of dry sand might mean oil accumulated at the bottom, wet sand might suggest another boring further up the slope of the formation.

A recent improvement in the method of drilling has greatly enhanced the value of

microscopical rock study in oil boring. Instead of cutting through the rock as a gimlet bores through wood, grinding the displaced material to powder, the method of "core" drilling gouges out a thin core or rod of solid rock. This preserves intact many of the inclusions—the larger fossils and the like,—that were crushed to fragments by the old method. Further, a piece of the core from each layer of rock passed through can be sectioned properly and thoroughly studied in all its points under the petrological microscope.

A study of the "well-log" of one of the famous American oil borings shows that in drilling down nearly 2,500 ft. no fewer than forty layers of rock, limestones, shales and sandstones varying from 10 to 280 ft. in thickness were successively cut through before the reservoir of oil was reached.

In modern oil-seeking, the geological character of the rocks encountered, their component minerals, fossil assemblages, peculiarities of structure and oil possibilities need careful study and trained judgment if the result is to be—oil. And an oil boring several thousand feet deep may be controlled to a large extent microscopically.

Coal.—The familiar coal of everyday use is a rock formed by a compact, stratified mass of "mummified" plants, mainly dating from the Carboniferous period of the world's history, when the predominant plants were giant ferns, horsetails and club-mosses. The scientific study of coal has engaged the attention of palæobotanists ever since the time of Witham, when the making of sections of hard material came into practice. Conjoined with chemical researches, it has sought to establish the constitution of the various types of coal, but the matter is not yet quite clear. Coal is a substance formed of the most diverse vegetable materials which have undergone great vicissitudes since they were first laid down; degradation products of the primary constituents have become incorporated along with these and complex chemical processes have taken place. Here and there individual tissues or parts of plants have been preserved and can be identified by the expert; but in spite of much patient labour, it is not yet possible to read the story of a slice of coal as the story of an inorganic crystalline rock can be read from a section. A promising recent development of technique has been the softening (by somewhat drastic means) of pieces of

coal to a consistency which allows of embedding and section cutting by microtome, as is done with fresh tissues.

When it is possible to interpret definitely the details of a microscopic section of coal, a great practical consequence will be the standardisation of coals according to their particular constituents. Everyone knows that coals from different localities burn differently; further, that there are several kinds of coal (ordinary bituminous coal, boghead, cannel, anthracite, etc.). There is some reason to believe that the varied behaviour of coals in burning depends on the proportion and type of the original constituent plants rather than on the chemical composition of the coal, for the chemical composition of plant-tissues in general does not vary greatly. The value of being able thus to grade coals accurately could hardly be over-estimated: it would ensure economical use of our coal resources which, though large, are limited, and it would be a boon to industry where the use of the right type of coal is often a serious practical problem. The problem has already been attacked by industrial microscopists. A case has been recorded of a gas plant using up 2,500 tons of coal a week. Careful selection of the coal

means much in the efficiency and economical running of a plant producing every week $3\frac{1}{2}$ million cubic feet of gas. The difficulty is that two coals may be similar in appearance and give nearly the same results on chemical analysis, yet one may be satisfactory and the other unsatisfactory in use.

Chemical Analyses of Two Coals.

	Volatiles.	Fixed carbon.	Total carbon.	Hydrogen.	Oxygen.	Nitrogen.	Calorific value. B.Th.Units per lb.
	%	%	%	%	%	%	
No. 1	38·2	61·8	82·2	5·83	11·21	0·76	14,750
No. 2	38·1	61·9	81·2	5·60	11·93	1·27	14,750

(Booth : *Jour. R. Mic. Soc.*, 1922.)

One of the above was used successfully in the gas plant already mentioned, the other could not be used economically. A practical problem thus arose of supplementing the chemical analysis by a test which would enable selection of the right coal for the particular purpose in view. It was solved by using the microscope in a comparative way. Sections of coals that had proved satisfactory and others that had proved unsatisfactory were made and kept for comparison with new deliveries, and it was found that coals with a

similar appearance—irrespective of what that appearance might mean from the palæo-botanist's standpoint—behaved similarly in practice. The method was extended to select coals suitable for " steam-boilers, direct-fired furnaces, coke manufacture, town's gas manufacture, etc."

This comparative method of coal selection proved very valuable to one of the biggest British munition firms during the late war, when restrictions were placed on long-distance transport of coal and the usual local supplies were insufficient for the increased demand. " As a consequence new sources of supply had to be tapped. Of these coals there was no previous experience, and careful selection was necessary, yet very many of them were rejected on microscopic evidence alone, little or no chemical analysis being done, and no large-scale tests run. This was a great saving of time to the laboratory staff. Though there were many anxious times due to traffic delays, etc., at no time was there any inconvenience caused by the fuel department being unable to supply suitable types of coal to meet the various requirements of the works. If deliveries of a certain class of coal were temporarily stopped, it was possible because of the more

detailed knowledge gained from the microscopic work to supply a similar type of coal, or else the nearest type available. This knowledge made it possible to put into immediate effect any alterations in the running conditions of the particular plant made necessary by the use of a different coal, and to issue instructions beforehand for any change in the working of the coal. Had the type of coal not been known any adverse effects could only have been combated as they arose. In some cases this would have meant delays for twelve to twenty-four hours, after the coal had been put on. It might even have meant partial stoppage and a consequent drop in production" (Booth, 1922).

We conclude this chapter by instancing one more field of activity where the microscopy of rocks is a valuable ally—the determination of the merits and demerits of various materials used for constructional purposes.

Experimental investigation of the properties of a rock is of course conclusive. But such tests for determining crushing strength, hardness, porosity, power of resistance to shock, durability under varied conditions, weathering qualities, etc., demand much time and costly apparatus. Microscopic investigation,

on the other hand, is speedy, inexpensive and in the hands of a competent person thoroughly reliable. The matter has been admirably summed up by an expert in engineering methods in the following words :—

" The first glance at a section of a rock through a microscope reveals the size and shape of the component minerals, the open or interlocked aggregation of the grains and the general texture of the rock. With a little patience it is possible to ascertain the several constituent minerals and their degree of alteration. All these little facts, when pieced together, provide sufficient data to enable an accurate opinion to be formed as to the suitability of the rock for various purposes " (Fox, 1923).

Let us illustrate. The value of a rock for road construction will obviously depend on its hardness and toughness to withstand the heavy wear of a road surface. Hence granite-like rocks of close texture showing hard minerals like quartz in equal-sized grains will be best : open sandstone, where the quartz grains are uncemented and not interlocked, is not suitable, as, being friable, it grinds to powder under heavy traffic.

In general, the strength and durability of a

stone can be gathered partly from the hardness or otherwise of the minerals seen in it and partly from the way in which the mineral particles are arranged; tightly packed grains firmly bound together make for a stronger and more lasting building than uncemented, even though hard, minerals in a porous rock. Texture thus points to strength, and in general it has been found that medium-textured varieties of rock are strongest.

The behaviour under heat of the principal minerals in the stone used is of paramount importance in the erection of fireproof buildings. The presence of quartz and felspar, minerals differing widely in their expansion under heat, may lead to enormous internal strain and consequent splitting when exposed to fire. Many granites are thus unsuitable. Similarly, marble of a kind found to contain equal proportions of calcite and dolomite cracks and breaks. Hard sandstone or quartzite, a firm, compact rock which on sectioning shows a mass of quartz grains bound together by a silicious cement, expands greatly when heated and loosened joints, bulging walls and collapse may be the result of its use.

Some of the constituents in rocks much in

favour for constructional work may from time to time be found showing decomposition. "The altered condition of a single constituent, *e.g.*, felspar in a medium-grained granite, may very seriously affect the strength and durability of the rock as a whole. Concrete in which the aggregate is granite with decomposed felspar, which has been used dry, will be subject to serious contraction. . . . A dam made of concrete with such ingredients is certain to contain cracks, which might result in serious loss by leakage and possibly in the collapse of the dam." To the trained eye this decomposition will at once reveal itself in a rock section.

We shall consider later on the application of the microscope to certain products of the earth's crust utilised in various industrial processes and manufactures. In this chapter we have sought to show the value of microscopy in solving the riddle of the rocks, and to illustrate its usefulness when this knowledge is turned to practical account.

During the recent war occasion arose to find out the nature of the raw materials used in a particular German cement. It was very desirable to know where these came from. Expert microscopic examination disclosed that

in making up the cement a certain volcanic rock had been used. This rock was known to be found in only one locality. Could one have a more singular instance of the fruits of geological microscopy?

CHAPTER V

THE MICROSCOPE AND THE WORKSHOPS

It is a well-known fact that during the last hundred years there has been a steady revolution in industrial practice throughout industry in general, involved in what is called "placing it on a scientific footing." Manufacturing tradition has been tested in the light of the results of science and the chaff separated from the wheat. Time-honoured empirical methods have given place to methods based on careful observation and experiment to ensure a high standard of product and to seek out improvements. There has been a getting to the bottom of things.

Applied science, as one of the greatest exponents of scientific method has pointed out, is simply the application of pure science to detailed practical problems, and hence it is not surprising that the tools of the scientific investigator are no longer restricted to the

college laboratory, but tend to find a place in the workshop of the industrialist.

It has been said that it would be easier to indicate the branches of industry in which the microscope is *not* used than to attempt to enumerate those which make use of it. This is an anticipation. The application of microscopy to industrial needs is of too recent a date to justify such a sweeping statement. The facts of the matter point to the conclusion that some industries of first-rate importance have reached a stage where microscopy is a recognised aid to progress. The steel industry is a good example. A large and increasing number more, such as the cloth and paper-making industries, have already learned the value of the microscope in practical working. Many others, rubber and constructional industries, are exploring its possibilities, and it is only a matter of time until the microscope figures in every workshop where modern methods are in use. The Royal Microscopical Society of Great Britain has reflected the trend of affairs in this direction by the formation of a special section for industrial microscopy.

An important factor in the extending application of the microscope to industrial

affairs is the number of skilled microscopists available. Every excursion of microscopy into industry calls for observers trained in knowledge of the particular sciences underlying the industry in question and equally conversant with its practical affairs. To this must be added a thorough knowledge of the principles of microscopy, and as yet there are few institutions where an adequate training in microscopy can be obtained. On the other hand, the parable of the seed sown is to some extent applicable to the microscope in industry, though this involves the assumption (also made in the parable) of the seed being everywhere capable of fruition.

We go back to the middle of last century—to Pasteur, master of microbes—for an early example of the debt of industry to the microscope. In 1853, disease broke out among the silkworms reared on an extensive scale to supply the raw silk for the French silk industry. The disease attacked, not only the silkworms (the caterpillars of the silk moth), but riddled every stage of the life-history of the insect, eggs, worms, cocoons and moths. Associated with it was the appearance of little dark spots which from their resemblance to grains of black pepper resulted in the

disease being called pébrine. Every attempt to combat it failed, and in five years' time the silk crop fell to less than one-third of its normal amount. Quack remedies were tried and failed by the score. So far from subsiding, the disease began to spread into other countries supplied with exported eggs, and half of France was wringing its hands in despair. Huxley, in his Presidential Address to the British Association in 1870, described the disaster in vivid language. "This means not only that the great number of people engaged in silk-growing are some thirty millions sterling poorer than they might have been; it means not only that high prices have had to be paid for imported silkworm eggs, and that after investing his money in them, in paying for mulberry leaves and for attendance, the cultivator has constantly seen his silkworms perish and himself plunged in ruin; but it means that the looms of Lyons have lacked employment, and that for years enforced idleness and misery have been the portion of a vast population which in former days was industrious and well to do."

Pasteur attacked the problem in 1865. For over two years patient microscopic studies and experiments, involving the examination

of thousands of specimens, and backed by one of the best brains in Europe, were steadily carried on and eventually crowned with success.

The disease is caused by a minute protozoon parasite—*Glugea bombycis*—which first shows itself in the vicinity of the food canal and thence spreads till the silkworm becomes one mass of parasites. Pasteur made clear two things: first that the disease was extremely contagious; second that the eggs of an infected moth were already doomed when they were laid. Success demanded that above all only eggs from non-infected moths should be reared and these safeguarded against contamination, especially in their diet of mulberry leaves. Each moth was placed on a clean piece of linen, on which the eggs were deposited. The moth dies, is dried and the dried body pounded up in a few drops of water. Some of this water is then examined microscopically and if parasites are found, then linen, eggs and everything else are burnt. If no trace of infection is visible the eggs are sound and may be reared. Pasteur laughed to scorn the idea that the silk cultivator could not use a microscope once he was shown what to look for, and he carried his

point. The net result was nothing else than the resurrection of a staple industry of France.

We again turn to Pasteur, whose investigations were stimulated by a profound conviction of the value of science to mankind, for the beginnings of the microscopical control of the process of brewing: "in which living ferments, bacteria and yeasts, play an important part either helpful or injurious to man's enterprise." Pasteur's studies on ferments led him to the conclusion that alcoholic fermentation was not a chemical process, as was then considered to be the case, but was due to the presence of microbes in the wine or beer. In 1871 he visited England and demonstrated this to the London brewers. In 1876 his "Studies on Beer" was published, and in it Pasteur emphasised the fact that if intruding bacteria are present unhealthy changes such as souring occur in beer: if, however, the beer is kept free from microbes such undesirable happenings are avoided and the liquor retains its qualities. This was a great step, and three years later Hansen followed it up by showing that besides bacteria the brewer had to contend with other microscopic adversaries in various kinds of undesirable yeasts which are present along with

the brewer's yeast and in the air. One of these, when present, gives to the beer an unpleasant taste and a disagreeable smell. Another makes it cloudy; a third produces a "marked fruity flavour."

On these discoveries rests the practice of

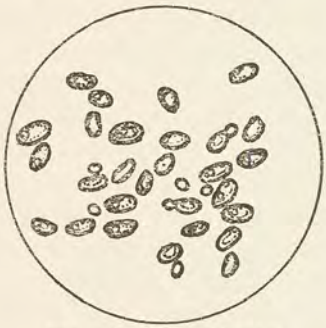


FIG. 18.—YEAST CELLS.

modern brewing, where the most stringent precautions are taken to avoid microbic contamination; in the plant, during the making and especially in the brewer's yeast. Brewing operations are conducted on such a scale that disaster would be extremely costly and hence constant and systematic testing of the pitching yeast goes on, to ensure it is free from bacteria on the one hand, and from

other yeasts of an undesirable kind on the other. In the laboratory attached to the brewery the selection and control of the yeast under the microscope is the key to the success of the industry. Many breweries will only use " single-cell " yeast, to ensure absolute purity. Single-cell yeast begins in the isolating of one cell of pure yeast, which is then grown or " cultured " free from all possibility of contamination to provide the required quantity. A very high authority has said : " The microscope is to the modern brewer what the compass is to the navigator. It is an instrument to which recourse is constantly being had, and without its aid the brewer, like the navigator without a compass, would speedily find himself on the rocks " (Chapman, 1922).

Textiles.—It has been pointed out that by the microscope alone we know that the shrouds of the Egyptian Pharaohs were made from linen material, while those of the Peruvian mummies were of cotton. This illustrates the essential service of the microscope in the manufacturing of fabrics, in the group of trades which clothes the world, the great Textile Industry. A distinguished writer has said that civilisation largely depends upon

the possibility of wearing cheap garments which can be washed; hence there are fifty million spindles in the spinning mills of Lancashire alone.

In the making of textiles the microscope is the means of investigating the component threads, whether in the state of raw materials, yarns or in the fabric itself. Both ordinary light and polarised light are employed. The raw materials are silk, wool, mohair, alpaca and other animal hairs; cotton, flax, jute and other plant fibres. Reference has already been made to the differences human hair exhibits under the microscope, and in the same way the various animal hairs can be tested and identified. Their thickness, pigment, the nature of the core and the cuticular cells on the outside of the hair are important characteristics. A very interesting example of how the microscopic structure of hair is bound up with its value for manufacturing purposes has been shown recently by the work of Professor Duerden and Miss Ritchie (1923) on kemp fibres in the merino sheep. It has long been known that in the wool of the merino sheep there occurred numbers of coarse opaque and brittle strands called kemps, which interfered with the uniformity

of colour and texture of the manufactured article, showing up as more or less undyed streaks and giving the yarn and the fabric a speckled appearance. Professor Duerden has showed that these kemps are the lingering remains of a tough outer coat well developed in the ancestors of the present merino, and which has not yet been quite bred out. His studies have shown that whereas wool is an elastic solid hair, without a core, kemp is a non-elastic hair which under the microscope shows a thick central core largely taken up with cavities containing air. The reflection of light from the surface of these air spaces gives kemp its opaque, white appearance, and though it actually dyes just as completely as wool the included air prevents the colour showing in its perfection, and hence kemp will not blend with dyed wool (containing neither core nor air) and produces a mottled effect in the fabric.

The examination of plant fibres shows the nature and condition of these substances as informatively as it does animal hairs.

When the cloth is woven the microscope provides the means of analysis of the constituents and of determining the nature of the cloth. The evenness of the weaving, the

texture, the number of threads to the inch of warp and weft become clearly visible, and whether the fibres are in their normal state or have undergone treatment which has altered them in any way, as in mercerised cotton. The different constituents present may if necessary be picked out by appropriate reagents and artificial silk is distinguished from real silk and cotton from linen.

A matter of great economic importance is the destructive action of various microbes (fungi and bacteria) on the vegetable fibres which form the basis of textile manufacture. During the war it was found that the raw cotton imported into Great Britain for war purposes showed 10 to 15 per cent. of " fly "—cotton fibres disintegrated and broken up into very short lengths. It has been found by Thaysen and Bunker recently that even in the best samples of cotton cellulose-destroying microbes are present, and that under conditions of moisture these attack the cotton fibres and disintegration results. Further, the resistance of Indian cotton to these minute enemies is less than that of cotton from America and Egypt. These two facts point the way to mitigate the trouble, by greater care in warehousing the cotton after

harvestry, so as to avoid wetting from rains, and by concentrating on the production of a highly resistant cotton. Susceptibility to destruction is not confined to raw materials, so the importance of keeping the manufactured fabrics free from damp is obvious.

There is a close resemblance between textile manufacture and paper-making in the part played by the microscope in these industries. Paper is a product where vegetable fibres are matted together, not woven, but its manufacture involves similar reliance on microscopic control. Paper-making was one of the earliest industries to utilise the microscope long before modern methods were in vogue, owing perhaps to the popularity of pieces of different papers as objects for the old-fashioned microscopes once found in so many homes. The introduction of various grasses and woods for paper-making instead of rags, about the middle of the nineteenth century, called for increased use of the instrument, and in no branch of industry is there a wider field, as Strachan and others have pointed out. Identification of plant fibres is the primary use of the microscope in the paper-mill, both in the analysis of raw materials, rags, wood pulps, straw, esparto grass, etc., and in " con-

trolling and checking the work of the practical paper-maker in his blending of various fibrous stocks." The sizing of the paper, where starches and other materials are investigated; the results of beating the fibres to minute shreds; the examination of clays and other "fillers"; the elucidation of impurities in the paper and in the water supply of the mill—all these come into the routine of paper-making microscopy. Then there is the constant search for new materials rich in cellulose.

One of the more recent industries to find virtue in the microscope is the rubber industry. It was a long time after the sailors of Columbus first learned the peculiar qualities of rubber that it became an article of commerce, but since the introduction of the process of vulcanising, less than a century ago, it has risen to the place of one of the world's most valuable industries, and the last two decades have seen the microscope more and more in use in various stages of its manufacture. There are two stages in the production of rubber articles: the preparation of the raw rubber as collected from the plant, and the making of this raw material into the manufactured article. The microscope is the means

of determining in the sheet and crêpe rubber of the plantation the causes and origins of various defects which seriously impair its value for manufacture. Minute air bubbles, included droplets of moisture, " rust " associated with the activities of microbes, impurities such as particles of bark of the rubber plant and other similar imperfections betray themselves under microscopic examination. For most manufacturing purposes, rubber is mixed with sulphur in greater or lesser proportions and with various pigments—carbon black, zinc and iron oxide, barytes, etc.—added in the form of powder. Certain aspects of these substances have to be very carefully considered in view of the marked effect they have on the finished rubber : in particular, the microscopic nature of the constituent particles, their uniformity of size and their dispersion through the rubber matrix. This last is studied in sections of rubber cut by a special freezing microtome. Rubber is a difficult material to section on account of its elasticity and sensitiveness to reagents and it is only recently that satisfactory methods have been evolved. Valuable fields have been opened up in the study of the appearances in rubber under strain, in the

investigation of the " perishing " characteristic of vulcanised rubber and the happenings in rubber during the process of vulcanisation. Sections cut from samples of rubber enable the detection of different organic substances which may be present, such as rubber substitute, glue and devulcanised rubber. The microscopic inspection of waterproofed fabrics, whether proofed by rubber or by bitumen, shows the extent of the impregnation as well as the nature of the material. Nowadays mixtures of rubber and bitumen are extensively used for making pneumatic tyres. Examination of sections under the microscope enables the quality of the product to be judged and faults in mixing and incomplete vulcanisation to be detected.

In every workshop one of the most important practical matters is the proper lubrication of the various kinds of machinery in use, on the efficiency of which the prosperity of the industry depends in no small degree. To ensure the best working of a particular engine or other machine, it is very necessary that the lubricant be exactly suited to it, and the selection of an appropriate lubricant is very often a difficult business. The microscope, however, allows the behaviour of a

lubricating oil to be gauged with confidence. Examination of a small quantity of the lubricant after it has been in use for a known time shows its condition compared with similar unused oil and whether it contains particles of foreign matter, especially metallic particles. Hence the rate of deterioration and its efficiency as a lubricant can be ascertained. Mr. H. B. Milner has recorded that during the war extensive microscopic examination of the lubricants used with captured enemy aircraft and other machinery was carried out as part of the routine work of the Aeronautical Investigation Department of the Ministry of Munitions. Besides lubricating oils, graphites and greases are in common use as lubricants. Many greases contain various substances : horsehair, cotton fibres, mica or talc, intended to give them a certain degree of consistency or to play a useful part in withstanding great friction. It is of the utmost importance to know the exact nature of these grease compounds, and this and the characteristics of the various ingredients are directly shown up under the microscope.

An industry which owes much to the microscope, and to which the microscope owes almost everything, is the making of glass,

which in this country and in America made great strides during the war. Owing to the stoppage of foreign supplies, we were compelled to make our own optical glass, and this is now the best obtainable.

One of the difficulties in glass-making is cooling the glass "metal" without internal strain, which very seriously interferes with the optical properties of the glass; and it is extremely important that any strain should be recognised early and its position located in relation to the surrounding material. Good glass is isotropic (singly refracting); but where internal stresses have been set up, the glass behaves in anisotropic fashion and objects seen through it appear distorted. This accounts for the disturbing effects produced here and there by cheap window glass. Recalling the *modus operandi* of the petrologist, we see that the polarising microscope offers a valuable means of judging and locating strain by the optical effect produced. A very interesting type of instrument has been devised for this and similar purposes. It is an adaptation of the Greenough binocular microscope, to which is fitted a polariser consisting of a piece of black glass, mounted at the proper angle to give a beam of polarised light

by *reflection*. In the two bodies of the microscope analysers are mounted. This arrangement, with paired objectives and eyepieces results in a polarising microscope with true stereoscopic binocular vision, great depth of focus and allowing a ready appreciation of the relation and position of the strain to the remainder of the material.

The biggest ingredient in all glass-making is sand, and the microscope is invaluable in the examination of sands, though for certain purposes it is necessary to supplement it by mechanical analysis. It " shows at a glance the nature of the component minerals of the sand, their relative proportions and the consequent purity or impurity of the material for the particular purpose desired. The size, shape, degree of angularity or otherwise and the nature of the cementing medium of the constituent particles of the sand are all rapidly appreciated by microscopical examination; similarly a comparison may be made between various samples submitted to manufacturers, and in this way standard grades differentiated from inferior grades, a check thus being kept on the uniformity in quality of selected material " (Milner, 1923). The examination is carried out by petrological methods. The

microscopy of sand has gone a long way since the days of Leeuwenhoek, whose use for sand was to place a single grain in the field of his microscope as a gauge of the size of his animalcules "by comparing them with the Bigness of a Grain of Sand."

A pure sand composed of angular or sub-angular grains of uniform size is desirable for glass-making. Its mineral composition must be precise, showing much quartz, very little felspar and iron and a minimum of accessory minerals. Apart from the manufacture of glass, sand is a material widely used in industry for the most diverse purposes, and the rapidity and the accuracy with which microscopical analysis can be made by a qualified petrologist indicate great possibilities for microscopy in the many different industries where rock products in one form or another are involved: the making of porcelain and other ware, glazes, silica bricks, paints, abrasive and cleaning powders, the selection of sand for moulding, the examination of agricultural fertilisers and tooth powders. And there are many more.

We may give a further instance. It has been said frequently that we live in the age of concrete. Even sculptors are fashioning

great groups of statuary from concrete; and everyone knows the concrete city of the British Empire Exhibition of 1924–25. Already the microscope has been applied as a critic of concrete and its important constituent cement. Sections of hardened cement are prepared by grinding in the same way as sections of natural rock and reveal a host of information; " directional properties, contraction effects, weak zones of cohesion, size and prevalence of voids, secondary chemical developments," and so on. Experimental investigations of this kind give the manufacturer positive evidence of the behaviour of his product and indicate any points in which alteration or modification of manufacturing practice may be desirable to improve its quality. Similarly, sections of concrete may be tested: this is particularly useful in the tracking down of the causes of defects which have shown themselves in the structure, and in determining the precise effect of weathering and other external influences.

The usefulness of the microscope in operations of great delicacy or precision is easily understood to everyone who has seen a watchmaker's eyeglass or used a reading glass—both simple microscopes. This form of employment for the microscope is widespread in

industry. When a process block such as is used in the reproduction of a book illustration is being made, a metal plate is etched or "bitten" in a shallow bath of etching fluid. The etching fluid eats away the outer surface of the metal to varying depths as required, according to the nature of the picture. It is most important that this etching process should be carefully controlled, and this is greatly facilitated by a specially designed build of microscope which allows every part of the plate to be examined as it lies in the bath, and permits the progress of the biting to be clearly seen and closely watched. At a higher level of precision is the work of measuring accurately small metal parts. A specially designed form of microscope is employed for measuring screw threads and similar objects. This very useful tool will allow of the measurement of the length and pitch of a screw to $\frac{1}{25000}$ in., of the diameter to $\frac{1}{2500}$ in. and of the angle of the thread to 5 minutes.

We come now to a branch of microscopy with its own special methods and apparatus, which has been of great worth to a large group of important industries: the microscopy of metals.

METALS.—This extremely important branch

of industrial microscopy began with the work of Sorby, who, we have already seen, was the first to discover the value of ground sections of rocks in geological study. From his study, in the 'sixties, of the structure of the meteorites, with their contained iron, Sorby was led on to study iron and steel. It was his great merit that he was able to appreciate the value of the examination of the minute structure of metals in various states (he was considered silly for suggesting the micro-examination of a rail whose breaking had caused an accident), and also that he devised the methods for doing this, which are now in everyday use in every metal and engineering works.

Before going on to consider the practical value of microscopic analysis of metals, it is necessary to understand how microscopic examination of pieces of iron, steel, brass and other metals is carried out. The fundamental difficulty is that even if sections be made and ground, as in rock section-making, they are still not sufficiently transparent to allow of examination by transmitted light. Metal specimens must therefore be illuminated from above. There are various ways of doing this, but the usual method employed is that of

"vertical illumination." A very thin piece of flawless glass—such as the coverslip for

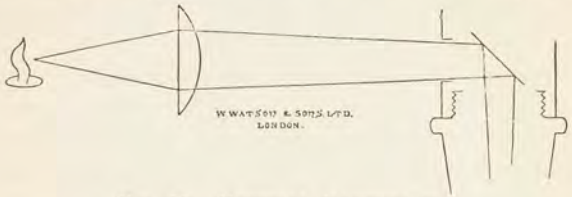


FIG. 19.—VERTICAL ILLUMINATION.

The light rays enter at the side of the tube and are reflected down on to the specimen by a thin glass slip.

a microscopic preparation—is fitted at an angle behind the objective, in the tube of the microscope. A beam of light is passed into

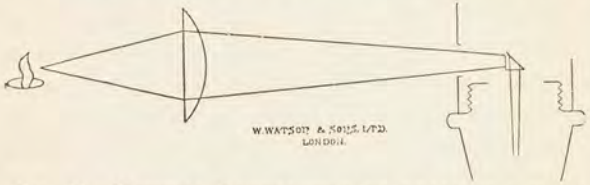


FIG. 20.—VERTICAL ILLUMINATION BY MEANS OF A SMALL PRISM.

the tube from the side and reflected by the glass slip down through the objective on to the specimen (see Fig. 19). The glass slip being perfectly transparent permits the specimen to be seen clearly. Alternatively, a

small prism takes the place of the glass slip, but this brings certain disadvantages in high-power work in that it reduces the numerical aperture of the objective and consequently its resolving power.

To allow the illuminator to be kept opposite the beam of light there must be no focussing up and down of the microscope tube, and accordingly this is kept fixed and focussing done by moving the stage whereon the specimen lies. No sub-stage apparatus is required, and in the metallurgical microscope its room is taken up by a rackwork, which permits the stage to be moved freely up and down. The metallurgical microscope is then, like the petrological microscope, a modification of the ordinary pattern suitable for the special work in view.

The specimen of steel or other metal is prepared by polishing the side to be examined. But just as the different tissues in a section of an animal gland or plant stem are shown more clearly by selective staining, so, as Sorby discovered, the constituents of a metal can be differentiated by attacking the smooth, polished surface of the specimen with an etching fluid. Etching fluids are chemical reagents—usually containing acid—*e.g.* 5 per

cent. picric acid in alcohol, which act selectively on the constituents of the metal causing differences of level or colour, and thus rendering the structure more distinct.

In certain cases other treatments, such as passing an electric current through the specimen in the etching-bath, heating so that different colours are assumed by different constituents and special polishing methods, bring about the desired result.

About the time that Sorby began the study of iron, Tschernoff showed the arrangement of iron and steel crystals in masses of these metals. It was some little time before microscopical investigation was appreciated as a great road to knowledge of metals and metal alloys, but following Sorby's pioneer work the last two decades of the century witnessed a gathering wave of enthusiasm which has swept steadily on to the present time. Martens in Germany, Osmond in France, Lynwood Garrison in America were in the forefront. In the main, work centred round the study of iron and its alloys and has since been extended to embrace non-ferrous metals. Microscopic investigation of the structure and the grouping of the constituents of alloys has gone hand in hand with

the study of their physical properties by thermal methods. The results in the metal and engineering industries have been profound. No works of any size is to-day without its metallurgical microscopes and apparatus for recording sections by photomicrography.

Metal microscopy or metallography is a field for the specialist, and we can but indicate here the main lines along which work is carried on. There is the microscopical examination of ores : there is the scrutiny of test pieces for faults in casting, blowholes, cavities and the detection of impurities of one kind or another. A faulty casting can be scrapped in time and the fault tracked down, without waiting till the material collapses in use. Then there is the control of the heat treatment of metal alloys, depending on a study of the minute structure. The great service microscopy has done to the metal industries is (1) that it has established the structure of metals and metal alloys. These latter are closely analogous in many ways to igneous rocks, in that both are the products of the crystallisation of mixed solutions. On the crystalline structure depend the physical and mechanical properties of the metal, and hence

its value for practical purposes. (2) The microscopic study of metal structure has established that structure (and hence mechanical properties) varies, not only in accordance with the constituents of the material, but also with the temperature at which it was cast, the time taken to cool and with the nature of the other operations, thermal and mechanical, to which it has been subjected.

One sample of steel, slowly cooled, will show a large, irregular structure, with hard and soft areas intermingled. A second sample, of high-speed steel, which has been overheated in hardening shows large crystals of " austenite " with oxide forming round them. Another sample, correctly worked, forged and annealed, will show a very fine-grained, uniform structure, evenly hard throughout. The two former will turn out mechanically weak or useless, the compact structure of the latter results in great strength and efficiency.

Thus microscopy provides an extremely valuable control in works practice, and a great power is put into the hands of the research worker in his search after new and improved alloys possessing definite qualities. Sir Robert Hadfield, speaking of the production of large calibre armour-piercing shells, has said : " We

could not really have obtained a shell of the requisite quality without the use of the microscope."

A natural consequence of the knowledge derived from a study of the minute structure of metals has been the employment of the microscope in the investigation of engineering failures, fractures, rail breakages and similar problems, involving the difficult question of metal fatigue.

Industrial Microscopy.—The use of the microscope in industry is so diverse that our examples in this chapter may seem to some to be detached and to have little connection with one another. This is necessarily so when our instrument deals with stuffs so wide apart as bitumen and baby foods, chrome steel and condensed milk. But certain general statements emerge, and we may try to sum up the *rôle* of microscopy in everyday industry. First, it avoids much unnecessary labour in selection of raw materials. Secondly, it is a means of delicate step by step control—a check—from the raw material right up to the finished product, of which it is the most exacting of judges. Thirdly, it promotes increased appreciation of detail, emphasises that it is the little things that count; in short, industrial microscopy

makes for a higher standard of manufacture in keeping with modern needs. If the developments of the last few years are a trustworthy guide, the future of industrial microscopy through the next few decades is exceedingly bright.

CHAPTER VI

INCREASE OF KNOWLEDGE

It has been said, and with truth, that of all the aids to scientific inquiry the one which has rendered the greatest services throughout science in general is the microscope. Utilised in the most divers kinds of researches, adapted in numerous ways to different types of work, it has become part of the stock-in-trade of almost every laboratory, and vies with retort and test-tube as the hall-mark of the scientist of popular fiction.

We may adopt a convenient division into Physical Science (Physics, Chemistry and correlated branches of inquiry) and Natural Science. As Natural Science are usually grouped Geology and Biology. Geological microscopy has already been considered in an earlier chapter, so that the second part, and the larger part, of our present subject will be concerned with Biology.

The atoms (to say nothing of the electrons)

of modern physical science are so infinitely small that it is obvious that no microscope can resolve them. But physicists have employed the microscope in a way which does allow us to perceive the presence of minute particles far below the limit of ordinary microscopical resolution. The most skilful use of the most perfect microscope may show as two separate lines a pair of lines ruled $\frac{1}{150000}$ in. apart. By the employment of what has been christened the "Ultra-microscope," particles such as those of colloidal gold which may be $\frac{1}{5000000}$ in. in diameter have been observed. Note, however, that "observed" does not mean "seen" in the sense that we see the size, structure or form of an object. Ultra-microscopical particles are too small to be seen, but their presence can be detected by concentrating a powerful light on them when they shine out and betray themselves, like the invisible motes in apparently clear air glisten when a shaft of sunlight penetrates the room. About twenty years ago the ultra-microscope was devised by Siedentopf and Zsigmondy and in the hands of the inventors, of Cotton, Mouton, Lord Rayleigh and others, has proved of great value in physical investigations. When

minute particles in a fluid or a gas can be rendered visible, although their structure cannot be made out, they can be counted, their movements followed and their behaviour under electrical and other influences noted.

The ultra-microscope is an apparatus consisting of a microscope in conjunction with a special form of illuminating apparatus. The latter is arranged to deliver a very powerful horizontal beam of light, which is concentrated against the specimen, which may be a solution perfectly clear and colourless under the ordinary microscope.

The depth or thickness of this beam, which is flat, not round, is of great importance. It is essential that the depth of the beam be no greater than the depth of focus of the microscope objective in use (which for a $\frac{1}{12}$ in. oil-immersion objective is very little indeed). Otherwise scattered light from outside the field of view would blur the picture. However, with correctly adjusted illumination, on looking down the microscope, we see, not a clear fluid, but a multitude of bright points against a dark background, all jostling one another and quivering in restless "Brownian" movement. Apart from the motion, our observation is similar to the observation

of the stars on a clear night through a telescope. The stars are too far away for us to see their size and structure, but they, too, advertise themselves by the light which they scatter, and when enough light is scattered, our telescopes can pick it up and we are aware of the star. In a sense most stars are "ultra-telescopic."

It is interesting to note that the molecules of certain substances, such as starch, whose molecule has been calculated to be rather bigger than $\frac{1}{5000000}$ in. in diameter, are within the limit of ultra-microscopic perception.

There is the physicist's use of the microscope for making measurements and kindred observations of great exactness. The astronomer uses the microscope for measuring stellar pictures in mapping out the skies. The great progress made by the photographic research chemist in studying the effect of light on the photographic plate is largely due to the facility with which the grains of metallic silver deposited on the development of the plate can be examined with the microscope.

In many instruments the microscope is conjoined with other apparatus with the

object of increasing delicacy of performance. Delicate measuring apparatus culminates in Dr. Tutton's comparator for calibrating the standard yard, where measurements are made in wave-lengths of light : the two microscopes employed are so adapted that each functions as an interferometer.

Towards the latter part of the eighteenth century, Marggraf introduced the microscope into the chemist's laboratory for examining the crystalline forms of different substances. Thereafter, great interest was aroused in the behaviour of various crystals in polarised light, observed by the polariscope or polarising microscope. Biot, Mitscherlich and others paid great attention to this, and Mitscherlich, noticing the similarity of crystals of phosphates and arsenates of potassium and ammonium, was led in 1819 to the discovery of the principle of isomorphism. This means, in a general way, that substances of similar chemical constitution form crystals of similar form. A discovery which, when followed up, has yielded important results in pure chemistry, especially in the establishment of atomic weights.

Another great step was made by Pasteur, whose microscopical activities were manifold,

when he introduced into chemistry the conception of molecular asymmetry, arising out of his famous studies on crystals of tartaric acid in polarised light. He came to the conclusion that molecular structure is not only of two dimensions but three, a conclusion that has meant much to chemistry. In the words of Professor J. F. Thorpe : " It is unquestionably the influence of Pasteur's work which led van't Hoff and, independently, Le Bel, to formulate the tetrahedral theory of the carbon atom, a theory which has led to the development of organic chemistry to a position which places it in the front rank of the exact sciences."

The widespread interest, in the middle of the nineteenth century, in the optical properties of crystals had other important results. It gave a great impetus to the study of mineral crystals which, as we have seen in Chapter IV, has proved very fruitful for mineralogy and geology. Further, it led to the development of new apparatus, including the developing of the polarising or petrological microscope to an instrument of high perfection. Crystallographers used light to investigate crystals. Crystals in return gave much valuable information on the nature of

light, and thus the way was paved for modern work on crystal structure by means of *X*-rays. Dr. A. H. Tutton has summed up admirably the place of the polarising microscope in crystal study. After referring to the development of special instruments, each designed for investigating one or another of the properties of crystals, he says that the microscope "is a sort of *multum in parvo* of them all; most convenient for preliminary work and the last resort in all cases where crystals cannot be procured of the necessary size (minimum, that of a pin's head) for individual measurement of each constant on its own specific measuring instrument."

The use of the microscope to the chemist is essential where only small quantities of a substance are available for testing or analysis. Poisons, and other substances on the nature of which much may depend, are examples. But Dr. Egerton C. Grey has gone further, and in his recent book on chemistry by microscopical methods, has shown how nearly all the usual operations of the chemist may be carried out under the microscope, with a fraction of the usual quantity of solid material, with drops instead of cubic centimetres of liquids. A great deal

of biological microscopy depends on microchemistry : the general technique of staining, the identification of blood crystals and the differentiation of fats in sections by polarised light are examples.

We see, then, that the microscope has been associated and is associated with a diversity of operations in physical science, generally as a valuable accessory, more rarely as the sole means of investigation. The case is different in biological science : the microscope is the biologist's main tool.

The place of the microscope in biology may be summed up very simply. Whenever the direct study of living things, in whole or in part, penetrates to the sub-visible level, the microscope is brought into use.

To say this may sound like " explaining the evident," but the application of the microscope in biological investigation is so wide that no narrower statement seems possible : it is a master key to the inner recesses of the realm of Nature. Thousands of species of living creatures cannot be seen at all save with a microscope. The biggest elephant or whale has phases of its life-history invisible except through the micro-

scope. The most massive tree has parts of its body which cannot be discerned by the unaided eye.

Biology is the science of life, and in the study of living things there are two closely related lines of inquiry. One is into their structure, the other into their working. What contribution has microscopy made to biology in these two directions?

It may be said at once that the *main* work of the microscope has been in the elucidation of structure, along several lines.

STRUCTURE STUDY: 1 (*a*).—If we take, say, a cat, examine it, measure and note its outward characteristics, then pass to the dissection of its internal organs—its muscles, its nerve trunks, its digestive organs, and so on —we are said to study the "gross anatomy" of our specimen, aided by scalpel and forceps and seeing things very well with the unaided eye in a good light. But if our specimen is a small creature like a bee or sandhopper, we need the microscope to see what we are doing. A low-power dissecting microscope is the thing; so we have the paradoxical state of affairs where a microscope is used in the study of gross anatomy. At a deeper level still, where smaller organisms like the

tiny worm *Filigrana* or some compound tunicates are concerned, gross anatomy merges with minute anatomy, ordinary dissection is difficult, unless we are of the tribe of Swammerdam or Lyonet, and we usually cut the specimen into microscopical sections and reconstruct the whole from the parts.

1 (*b*).—Minute anatomy or histology is the study of the tissues of animals and plants. In gross anatomy the use of the microscope is, in a way, subordinate. In histology, it is indispensable, for the structure of tissues of the very largest creatures is rarely visible to the unaided eye. A big slice of biological microscopy is therefore concerned with tissue study. Bichât, professor of anatomy in Paris, 1797–1801, set it on its way. The discovery, by 1839, that all tissues are made up of units of living matter—cells—gave it a firm foundation and biology had to take account of a deeper analysis of organisms than before—an analysis into the tissues of which the organs of the body are built up. Let us consider, of necessity briefly, what histology has shown regarding the animal body; first pointing out that there has been a similar study of plant tissues, which are ultimately composed of the same living stuff as animal tissues,

namely, protoplasm. The unit masses of protoplasm which make up tissues, the bricks in the building, are called cells. A tissue is a group or assemblage of cells which, being required to carry out a definite function, is specialised structurally in a distinctive way. Thus muscular tissue is an aggregate of muscle-cells, all of which are specially contractile and hence exhibit structural peculiarities in shape, cell contents and disposition of parts.

Cells are protean in size and shape. The egg-cell of mammals is barely visible to the eye, the egg-cell of a bird may be two inches in length. Some nerve-cells are but $\frac{1}{5000}$ of an inch in diameter. A human red blood-cell is one and a half times, a frog's blood-cell three times this size. Again, some of the giant nerve-cells of mammals reach from the cortex of the brain far down the spinal cord. The shapes are equally varied. Frog blood-cells are a clear oval, liver-cells polyhedral, muscle-cells typically spindle-shaped, nerve-cells like a star. The living jelly of all cells almost invariably shows a primary differentiation which allows us to perceive two structural features in cells : the cell-body (cytoplasm) and a more or less round body

within it, the nucleus (karyoplasm). Each of these has a variable number of other structures associated with it, both living and non-living. Thus in the cytoplasm we may from time to time distinguish centrosomes, mitochondria, "Golgi apparatus," fat globules, yolk platelets, pigment, etc.; in the nucleus, a refractile network (linin), chromosomes, one or more nucleoli. The nucleus is not invariably round. Long, spindle-shaped muscle-cells have an elongated nucleus, white blood-cells may show it a rugged U-shape, the protozoon *Stentor*, the trumpet-animalcule, has a nucleus like a string of beads. Round the outside of the cell the cytoplasm usually condenses to form a fine semi-permeable cell-membrane. (In plant-cells there is a thick wall of cellulose.)

The simplest animals, being unicellular, cannot be said to have a "body," but directly we come to the level of body-making animals from sponges onwards, examination shows that body to be built up of more or less well-defined tissues. In sponges and cœlenterates the tissues are not fully differentiated but in the worms, molluses, insects, crustaceans, spiders and other backboneless animals, and in all backboned animals the tissues are

of well-defined sorts. Four main classes of tissues are found. Muscular Tissues (or tissues of motion), Sensory Tissues, Glandular Tissues and Supporting Tissues.

Muscular tissue of the highest type is made up of a mass of muscle fibres each a long, spindle-shaped muscle-cell with the greater part of its cytoplasm differentiated into parallel, thread-like muscle fibrils, and possessing an oval nucleus pressed to one side of the cell by the mass of fibrils. These fibrils are the contractile elements of the fibre and in very quickly contracting muscle the fibres have a banded or "striped" appearance, due to the constituent fibrils showing throughout their length alternate highly refractile and less refractile regions. Such striped muscular tissue is seen typically in the wing-muscles of insects and wherever rapid muscular action is needed. For instance, in Crampton's muscle in the eye of the swooping bird, where exceptionally quick "accommodation" is necessary. Unstriped muscular tissue means less rapid contraction, as in sponges, worms and in the bladder of mammals. Special histological methods (impregnation with gold or silver) show that fine twigs of nerves end on the muscle-fibres.

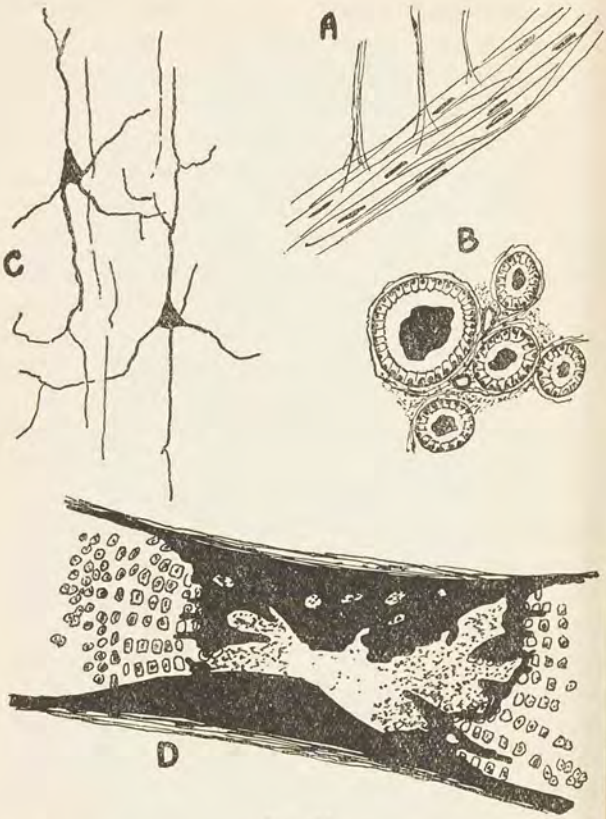


FIG. 21.

A. Smooth muscular tissue in the arm of an octopus. B. Thyroid gland of the skate (secretion, black). C. Branching nerve-cells in the forebrain of a cat. D. Bone replacing cartilage in an embryonic finger (bone, black).

The nerves control the contraction of the fibres. In some lowly creatures like the freshwater *Hydra*, differentiation of muscular tissue is not complete and fibrils develop in the roots of the layers of cells forming two-layered body-wall. In the nematode worm *Ascaris* found in the intestine of the horse the fibrils are massed to one side of the cells: with the result that the rest of the cytoplasm with the nucleus forms a balloon-like mass on the inner side of the fibre midway along its length. This condition is seen in an exaggerated form in the muscle-fibres of certain larval flatworms, where the fibrils are almost entirely dissociated from the rest of the cell, which is linked to them by fine protoplasmic processes.

Sensory or nervous tissue is made up of two kinds of cells, neurones and neuroglia cells, the latter being somewhat in the nature of supporting or packing cells for the neurones. A neurone is a cell of variable size, some being very small, others so large that minute blood channels pass through them. The nucleus is usually large and spherical with a well-marked nucleolus. The cytoplasm of the cell is drawn far out into long processes which leave the cell-body in one or more

directions: in these processes are differentiated nerve-fibrils. One of the processes is stout and prominent and is called the axon or nerve-fibre. A thin sheath covers the fibre. In most of the higher animals an inner coat—of fatty substance—is present in addition, and the fibres are termed medullated nerve-fibres. It takes a great many nerve-fibres to form a nerve-bundle, and several of these nerve-bundles, each in its sheath, are wrapped together in a mass of connective-tissue, and form a "nerve." The other processes which leave the nerve-cell are dendrites, which often branch extravagantly. In a typical neurone, it is the business of the dendrites to pick up or perceive a stimulus or message (either directly or indirectly). The stimulus is conducted *via* the cell-body along the axon and handed on to another neurone, whose dendrites or cell-body are in intimate association with the slender fibrils in which the axon of the first neurone ends.

Among the more lowly animals, nervous tissue is scattered and small in amount; in *Hydra*, for example, it forms a delicate network under the covering layer of cells, with here and there an odd sensory cell reaching

the surface. As we proceed up the scale, there is a concentration of nervous tissue toward the front end of the animal (*e.g.*, the earthworm) correlated with the habit of moving in a definite direction; at the same time, there is a marked stability of arrangement of nerve-fibres coupling up different parts of the body with the main nervous system. Eventually in backboned animals we reach a state where a central nervous system—brain and spinal cord—is linked by nerve-fibres to a peripheral nervous system extending to every corner of the body. The complexity of structure found in the nervous tissues, of the brain and some sense-organs especially, is extraordinary. The neurones of the cortex of the forebrain of mammals (to the number of several thousand million) form a maze of untold intricacy. Let us attempt to describe some of the associations of the optic nerve which leaves the back of the human eye and is conveying at this moment a latent image of this page to the reader's brain. The optic nerve leaves the eyeball after ramifying in the retina. The retina lines the wall of the inner chamber of the eye. Dissected out of the eye, it appears a thin, filmy membrane, more or

less structureless. If a section be cut across the thickness of this membrane and studied under a high power of the microscope, apparent simplicity gives way to a densely-packed mass of cell-bodies and granules arranged in eight layers or belts. Special staining methods (impregnation with silver) bring out the fact that these belts are largely nervous tissue, bound together by strong fibres passing through them. On the inner surface is a narrow layer of delicate fibres of the optic nerve. These are part of large neurones whose cell-bodies form a second belt. In the next belt the dendrites of these neurones link up with the axons of a second smaller set of neurones (4th belt). The dendrites of these in turn join up with axons (5th belt) of a third set of neurones (6th belt) whose terminal portions are known as rods and cones (7th belt) the ends of which lie in a pigment layer backed by a copious blood supply. And there are yet other elements in the retina.

It is essentially a characteristic of glandular tissue that it secretes. That is, it takes up something—some raw material—from the blood, transforms it or manufactures it into a definite product, mucus, gas, salivary juice,

etc., and then gets rid of it and usually proceeds anew to make some more. The getting rid may mean simply that the circulating blood absorbs and carries off the products as in the case of the important endocrine or ductless glands (thyroid, pituitary, etc.), or there may be a definite tube or duct specially to carry off the material secreted. Thus ducts from the salivary glands open into the cavity of the mouth; the minute ducts of the glands (including the tear glands) associated with the eye in higher animals pour out a watery secretion which keeps the eyeball moist. The variety of products that glandular tissues secrete is extraordinary: very many glandular tissues are associated in the digestive system of animals: some glandular tissues produce poison, as in some snakes; some produce lubricants, as in the skin of worms and the preen gland of birds; others manufacture strong-smelling fluids, as in the stench glands of the skunk and various bugs; others, again, make silk or material for the shells of eggs.

If we examine a section of the "ink bag" of a cuttle-fish under a low-power objective, we see a pear-shaped organ composed of a mass of little pockets or pouches. Under

higher magnification each of these is seen to be lined with glandular tissue engaged in manufacturing ink or "sepia." This tissue is composed of a layer of cells, of a low columnar shape, appearing in the section like a row of bricks set up on end. A large oval nucleus lies toward the lower end of each cell. The cytoplasm shows faintly granular. As secretion begins the cells grow larger, their free ends elongate and become less regular in shape and scattered grains of dark ink appear in the cytoplasm and gradually accumulate at the free end. Steadily the secretion goes on and eventually the whole of each cell down to the level of the nucleus bulges with a mass of pigment. Then the outer end gives way and out into the pouch bursts the accumulated ink, leaving a worn-out tissue behind, made up of cells containing only a nucleus and a very little cytoplasm. (In this particular instance the cells do not recover but disintegrate and a fresh glandular pouch carries on the work.) The accumulation of ink in the pouch fills the latter to bursting point and the ink passes out to the neck or top of the ink bag, there to be shot out on occasion into the water as a kind of smoke screen under cover of which the

cuttle-fish makes good his escape from pursuit.

The other group of bodily tissues, the Supporting Tissues, protect, bind together, or support the various parts of the body. Covering the free surfaces of the body generally is epithelium. Filling in the chinks, developing tough fibrils in the cells and forming strong envelopes and flexible scaffolding for various organs, or again forming fibrous tendons are different forms of connective tissue. More rigid are tissues whose cells surround themselves with a hard or resilient mass in which they lie as if embedded in a matrix; like the cartilage which supports parts of the body of some cuttle-fishes and forms an internal skeleton for "gristly" fishes like the skate and shark. In higher animals cartilage is to a lesser or greater extent replaced by a skeleton of hard bone. Cartilage or gristle shows little groups or rosettes of cartilage cells in a clear hyaline ground-mass. By studying the structure of successive phases in the gradual replacement of cartilage by bone which goes on for example in the limb bones of a very young mammal, a fascinating chapter in histology is unfolded. At first the whole

bone is " roughed out " or sketched in cartilage. When bone formation sets in a thin layer of bone, called perichondral bone, is formed on the outside from the overlying connective tissue. Changes of a most marked kind take place in the cartilage. At each end the disposition of the cartilage cells is normal; a little nearer the centre they arrange themselves in columns parallel to the length of the bone, and begin to swell up. Nearer still, the columns of cells start to disintegrate and their nuclei become ragged. At the centre they break up completely and the cartilaginous matrix round them shows signs of degeneration: here begins the invasion of bone (see Fig. 21, D) and proceeds from the centre outwards until the whole of the original cartilage has been converted into bone (endochondral bone). A blood-vessel loop grows in from the surrounding tissue bringing with it two kinds of cells; small bone-building cells, osteoblasts, which take up lime salts from the blood and deposit them as bone; and large osteoclasts, giant cells with several nuclei, which eat up the broken-down cartilage matrix. Blood-vessels and osteoblasts penetrate the spaces left by the cartilage cells and bone is laid down as they go. Osteo-

clasts clear away the *débris* as the invading army marches steadily on, replacing the original cartilage as it goes and leaving behind a ragged framework of bone which is eventually built up and consolidated. Numbers of osteoblasts are left on the way and become imprisoned in the finished bone. A finished bone seen in cross-section shows numbers of fine canals which run longitudinally through the bone—Haversian canals. Round these are concentric layers of bone; between the layers are minute spaces containing the bone-cells. Delicate channels link up the cell spaces with the central canal, which contains blood, nerve and other cells, and this permits nourishment of the imprisoned bone-cells, which thrust out slender processes down the canaliculi. The whole structure, Haversian canal, bone-layers and bone-cells is called an Haversian system and there are very many of them in a large bone.

In the study of tissues emphasis was bound to shift bit by bit to the closer study of the particular kinds of cells which compose them, and this is the main line of research in histology to-day. Cells are both the units of structure and of function in living creatures, and the study of them, which demands the

finest microscopical apparatus and the most skilful use of it, is of sufficient importance to need treatment by itself, and accordingly we shall return to it in the following chapter. In the meantime we may ask what has been the value of the study of minute anatomy of animals and plants by the microscope? As far as such a question can be answered we may say first the revelation of the extraordinary complexity of structure of living creatures. Secondly, the great contribution which microscopical anatomy has made to the other branches of biological study. At a very early date it stimulated the study of development which it partly overlaps; it had a profound effect on the study of disease; especially has it been of importance in physiology, where Cajal's great work on the histology of the brain and spinal cord—a most difficult field—has laid the foundation of our modern knowledge of the nervous system.

2. Our next branch of structure-study is the classification of animals and plants, on a basis of structural resemblances, Taxonomy —to give it its technical name. Here features of both gross anatomy and minute anatomy may have to be taken into account: to sort

out into phyla, classes, orders, sub-orders, families, genera and species, either plants or animals. Of animals alone there are probably over 600,000 species known to-day. Three-quarters of them are insects, a vast number of which approach the sub-visible level.

The classifier's work is based on the investigations of scores of specialists, one an entomologist, another a parasitologist, another an ichthyologist, an ornithologist, and so on. Nearly everyone needs to investigate the minute anatomy of his specialism and many from the nature of the subject are wholly dependent on microscopic study. The student of the simplest animals has a run of some 4,000 species of protozoa all of microscopic dimensions. The investigators of bacteria, unicellular plants, zoophytes, mites, ticks, rotifers, the lesser worms and crustacea each works in a microscopic world of his own. The microscope has meant enormous progress in orderly classification, in the working out of relationships. Its deeply penetrating analysis allows of the nicest discrimination in regard to species, deep down even to the numbers of chromosomes characteristic of the cell.

3. In the study of the structure of ancient animals and plants—the ancestors of our

present-day types—which are known only in the form of fossils, the microscope can, gener-

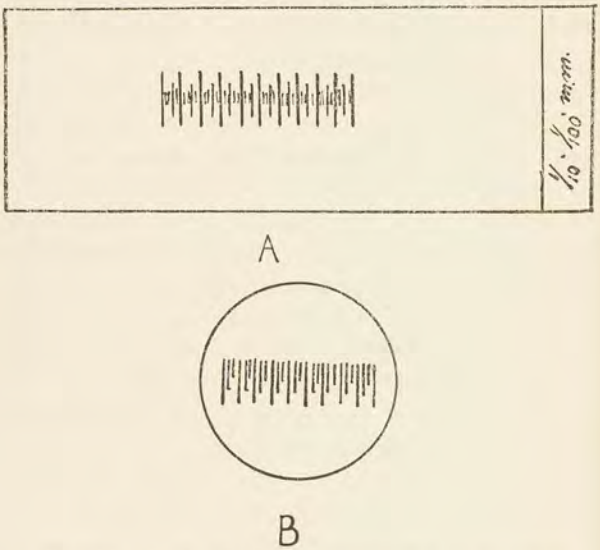


FIG. 22.—A. STAGE MICROMETER WITH FINE RULED SCALE. B. EYEPIECE MICROMETER.

The latter is carried in the eyepiece for measuring objects in the field of view. It is calibrated for different objectives by the stage micrometer.

ally speaking, be employed less constantly. All the soft tissues of fossil animals are gone (unless in the case of the frozen mammoth) and only a few types of microscopic animals

form a hard skeleton; the single-celled Foraminifera are an example, and a recent paper by Heron-Allen and Earland describes over two hundred kinds found in the "Filter Quarry," Moorabool River, Victoria, Australia, and dating from Miocene times. In regard to plant fossils, we recall that the discovery by Nicol of a method of making sections of fossil wood had great results in the microscopy of rock structure, and the method has led to valuable knowledge of the nature of one-time plants. Especially has it shown how old are the foundations of plant organisation. Professor Seward writes: "Microscopical research has demonstrated the remarkable antiquity of the architectural basis of plant organs. In the distribution of their vascular tissue, in the structure of the sporangia and other organs, the plants of former periods differed more or less widely from those of the present day; but in the construction of the unit of plant structure—the cell in its various forms—as also in the principles underlying the disposition and structure of the tissue concerned primarily with giving rigidity to sub-aërial organs and power to resist the force of the wind there is a remarkable agreement between the plants of the Palæozoic era and those which are alive

to-day." So we find the stomata (minute openings usually on the underside of the leaf by which carbon dioxide reaches the tissues) on the leafless stems of the Mid-Devonian genus *Rhynia* closely resemble those of modern plants : and the spore capsules of *Hornea* of the same age are like those of a modern bogmoss.

4. A field of biology where microscopy ranks high is the study of the structural appearances presented in " the long series of changes by which, as they melt the one into the other, like dissolving views the little white opaque spot in the egg is transformed into the feathered living active bird," as Sir Michael Foster put it. This study, whether of bird or of any other animal or of plant, is what is usually termed Embryology—the study of development—and has ever been a source of wonder from the days of Aristotle onwards.

Thanks to the labours of a multitude of microscopists, we now know the course development takes in a wide range of backboned animals of all classes, and in representatives of most of the groups of backboneless animals. Embryologists work largely by means of thin sections. Embryos are cut into hundreds of consecutive slices, spoken of as serial sections.

These are studied with the microscope direct; or, maybe, from the sections an enlarged model is built up in wax or with plates of glass. This is especially valuable in tracing the development of a complex organ or system of organs; models of whole embryos obtained in this way, and dexterously contrived to take to pieces and put together find another and valuable use in the teaching of students in our colleges and universities. Had the Chinese emperor, Liang Shao Pao (A.D. 980), some such idea in mind when he sent his physician with an artist to make anatomical sketches during an execution of thieves by the slow slicing process!

It is a familar fact nowadays that most animals start life as a single cell, formed by the union of a mother egg-cell and a fertilising father sperm-cell.

Curiously enough, it was not till the 'sixties that this statement could be said to have been definitely proved true. Though the general nature of fertilisation had been known for some time, only in 1865 was it recognised that the sperm was a cell like other cells. Not very long before, it had been counted an internal parasite of the male. In 1861, the egg-cell of mammals was discovered, and later

those of backboneless animals and plants. So that the observation of the Edinburgh student, Martin Barry, who, some twenty years earlier, had actually seen under the microscope the fertilisation of the rabbit's egg, was clearly explained as the union of two cells, male and female, forming a zygote—a single cell, the beginning of a new individual.

FIG. 23.—THREE STAGES IN THE EARLY DEVELOPMENT OF THE FROG.

4-Cell stage. 8-Cell stage. Blastula.

Development traces the changing structure of the young living thing from the zygote to the adult form—and does it stop even there? The zygote divides and redivides till a ball of cells is built up and we recognise a blastula stage. The transformation into a two-layered condition marks a second stage, the gastrula. These two stages are common to most animals, and are well seen in animals like the sea-urchin and lancelet. Thereafter

comes a three-layered stage, wherein the three primary layers of the future body—ectoderm, mesoderm and endoderm—become recognisable. Then follows the laying down of the chief systems of organs of the embryo, a process of differentiation from the primary layers, the more complex parts such as the central nervous system showing first.

In a developing mammal the ectoderm forms outer skin, brain, spinal cord and sense organs; the mesoderm the skeleton, muscles, kidneys and reproductive organs; the endoderm forms food-canal, lungs and liver, and the embryo gradually takes on recognisable form. By continued differentiation from this rough model the final form of the various organs and tissues is elaborated. Along with differentiation has been integration, the young animal has become more of a whole, a unity. Both processes eventually reach a point where the embryo can function as an individual, and accordingly, the hitherto intimate bond between mother and young gives way and the young mammal is born. Continued increase of size and the maturing of reproductive organs bring it to adult stage; and then, be it noted, the once unicellular zygote has become a creature

very like its parents. During the earlier stages of its development, certain curious happenings demand special notice. Gill-slits like the gill-clefts of fishes, appear in the throat region and disappear; a notochord appears and is gradually replaced by a backbone. What is the meaning of these transient structures? Looking round the animal kingdom generally, we find development to be a much chequered process. The cœlenterate gastrula becomes a planula larva, swims about by cilia and then settles down on a stone to grow into adult form. The frog embryo hatches out as a water-living tadpole with the heart and circulation of a fish and ultimately becomes an air-breathing frog. The chick inside the egg has its transitory gill-slits (though useless) and for a time its notochord; structures which are obliterated by the time the adult stage dawns.

These facts are inexplicable save in one way —that the developing individual recapitulates in some measure the history of his race. The tadpole represents a fish-like stage in Amphibian history; the rabbit's notochord is a legacy from primitive vertebrate times, when a notochord was the main supporting axis of the body, as it is in the lampreys of to-day.

Let us notice then, that Embryology, so

largely dependent on patient microscopic study, shows that in development the whole body, however large the animal, is built up by repeated division and differentiation of a single fertilised egg-cell or zygote. The study of successive stages brings to light the fact —of great moment to the biologist—that development is very frequently a circuitous process with many apparently superfluous steps and some short cuts.

Thus far we have attempted to show some of the results of the microscopic study of structure. It is now necessary to say something about the complementary side of biological inquiry, the study of function. As such we include the study of the working of the animal body, of the activities and interrelations of organisms generally, and of their becoming—their evolution.

In the main, the microscope contributes to the understanding of function through its revelation of structure. Thus we cannot understand the physiology of digestion unless we know the minute structure of the food canal, nor the circulation of the blood without a knowledge of capillaries and intravenous valves. Kulchitsky has recently shown (1924) that in the muscles of some backboned animals

there occur striped fibres of two kinds, one slender the other stouter. The large fibres are

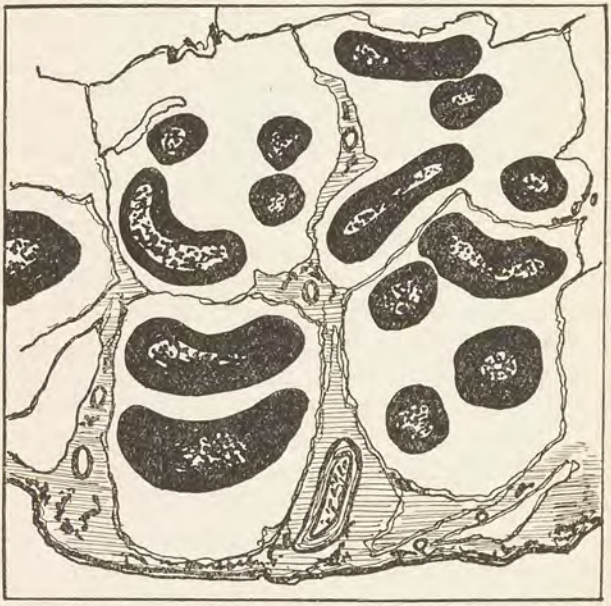


FIG. 24.—PART OF A SECTION THROUGH A FROG'S LUNG HEAVILY INFECTED WITH PARASITIC WORMS. (PARASITES IN BLACK.)

innervated by nerves from the central nervous system, " medullated " nerves ending on the fibres in prominent end plates. The slimmer

fibres are controlled by fine non-medullated nerves from the sympathetic region of the nervous system, with grape-like endings. This discovery leads on to interesting physiological developments. It is possible that the thick fibres are concerned in active movement of a voluntary or reflex nature, while the function of the thin fibres is in supporting the weight of the moving part and maintaining the position attained as a result of the movement.

Microscopic inter-relations form a considerable and instructive chapter in Natural History. Some of them are of amazing intricacy: ranging through all grades of relationships, from associations on more or less level terms like the algæ symbiotic in the outer protoplasm of radiolarians to a variety of one-sided partnerships, often with a grim touch, where parasitism is involved. "It seems hardly too much to say that the system of animate nature would be uncomfortably magical if the microscope had not enabled us to detect the missing links in many a chain of events." Starting from microscopic examination of minute hooks and suckers, Kuchenmeister worked out the life-history of the pork tapeworm and elucidated a threefold linkage between man, tapeworm and pig.

An interesting case has been reported from the Museum of the University of California. A pair of specimens of the plain titmouse obtained locally showed the under surface of the body bright yellow. These birds are normally of a light ashy-grey colour. Microscopic examination showed the feathers covered with masses of minute yellow bodies " uniform in appearance, elliptical, with a groove in one side, and with the surface finely speckled or ' sculptured ' " (Grinnell), and measuring about $\frac{1}{5000}$ in. in length. Was it a case of pollen-carrying by birds? Expert opinion finally diagnosed spores of a certain order of fungi. Titmice and fungi both haunt the hollows in tree-trunks, and the latter may from time to time benefit by their bird visitors, in securing a wide dispersal of the spores; though the association does not seem to have been regularised, or more yellowed titmice would be found.

Lastly, microscopy provides valuable evidence concerning the evolution of living things. From the study of development comes the knowledge that the fertilised egg-cell gives rise to all the cells of the adult organism, which in its turn liberates one of them, an egg- or sperm-cell, to start a new generation. Thus an organic continuity is established from genera-

tion to generation. Then development often takes a roundabout path with steps only intelligible as reminiscences. These, cautiously interpreted, hint back to bygone stages in evolution. The beautiful little " slow worm " (*Anguis*) is a limbless lizard; but there are microscopic limb buds in the embryo, and it is safe to say that its ancestors had legs like other lizards. Again, how many homologies, pointing to common descent, must be settled under the microscope?

We have referred for illustration and explanation in the foregoing pages chiefly to animal life; but biological microscopy is a boundless field and, did space not preclude it, nearly every instance might be duplicated from plant study.

CHAPTER VII

THE NATURE OF LIFE

It is now about fifty years since biological microscopy entered on its most subtle phase: the investigation of the cell. This was the outcome of the Cell Theory of Schleiden and Schwann (1838–39), a theory which is no longer a theory but enumerates some of the most fundamental facts in modern Biology.

Briefly, the cell theory laid down that the cell is the unit of structure and of function of all living things, plant or animal: and that all living things, reproduced in the ordinary way, begin life as single cells, from which single cell, in body-making animals, the body is built up by repeated divisions.

The consequences of this epoch-making generalisation have been profound. It showed a fundamental unity in the diversity of living creatures, and demanded a re-examination of the whole of biology from a new point of view.

A testing period ensued : in the light of the new idea development took on a new interest ; the activities of cells under both normal and abnormal conditions began to be investigated, and biologists turned to a closer study of cell structure. By 1870 the foundations of modern cell study had been laid and it had been shown conclusively that the egg and spermatozoon are both cells ; that all cells in the body arise by division from pre-existing cells ; and further that all cells, and hence all living things, plant or animal, are formed from a fundamental universal living stuff, called *protoplasm*. It became clear that the nature of life must be sought in the cell and the foremost workers in biology settled down to work at a deeper level than before, to study the cell in all its aspects. On the technical side, there were big steps in the introduction of staining to pick out otherwise indistinguishable elements in histological preparations and in the invention in 1870, by Wenham, of the oil-immersion lens, which placed a new power in the hands of microscopists.

It is important to remember that in the study of cells the microscopist is dealing with the *very* minute. At first sight this statement seems negatived by the fact that amongst the

reproductive cells of animals—the germ-cells —which have attracted the great majority of workers on account of their important biological bearing, there are some, like the eggs of sharks and birds, which are amongst the largest cells known. These are exceptional in size and the amount of protoplasm they contain is extremely small, the main mass of the egg-cell being taken up with food yolk. More typical are the eggs of other fishes and amphibians, where a quite yolky egg is but $\frac{1}{20}$ of an inch in diameter. The less yolky eggs of mammals are still smaller, frequently one-tenth of this size. The male germ-cell or spermatozoon in most animals is of extraordinary minuteness. Human spermatozoa are slender cells $\frac{1}{500}$ in. long and the greater part of the cell, even under the highest power of the microscope, appears of the thickness of a delicate thread.

Such are the dimensions of the elfin world to be explored. Cell study, therefore, calls for the very best that can be got out of the microscope, particularly in the use of oil-immersion objectives of maximum resolving power, and worked in conjunction with an adequate and correctly adjusted condenser. It may be pointed out that it is misleading to use with a first-class objective of N.A.

1·4 a condenser whose maximum aperture may be 1·0 but whose aplanatic aperture (flat field) is only 0·5. This is hardly universally appreciated, and undoubtedly accounts for many of the discrepancies which have occurred from time to time in the observations of different workers in a difficult field.

Realising, then, the delicacy of the operations, let us see what kind of results have been achieved in this branch of microscopy—cytology, to give it its technical name—and notice how they are bound up with the deepest problems of Biology.

Cell Structure.—We have previously indicated some of the characteristics of cells and mentioned some of the parts of a cell. A very good example of a typical cell round in shape is the egg-cell or ovum of a rabbit or cat (Fig. 25, E). We may imagine a living cell as a bladder filled with semi-fluid substance; within the bladder, poised in the jelly, a second small bladder, similarly filled. The whole formed of protoplasm in which movement indicative of ceaseless chemical change is going on. "A cell is a bit of protoplasm containing a nucleus."

The nucleus of a resting cell shows a mass of very fine network extending throughout it.

In the meshes of this linin net are knots and fragments of denser material, which has a particular affinity for certain stains and hence is called *chromatin*. A small round body, usually lying in the nucleus, is the nucleolus.

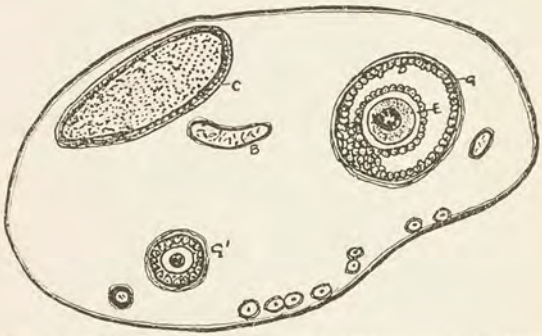


FIG. 25.—SECTION THROUGH THE OVARY OF A RABBIT.

E Egg-cell. G. Graafian follicle. G'. A younger follicle with egg. B. Blood-vessel. C. Follicle from which egg has been discharged. A number of very young follicles are seen along the lower edge of the section.

The chromatin is a very important part of the cell, as we shall see presently; in fact, for a time, there was a tendency to consider it as the only part of the cell deserving of study, but latterly this extreme view has been reasonably modified. However, the study of the chromatin in its various phases has been one of the

most marked and most valuable branches of cell study.

In the cytoplasm, which is sometimes vacuolated, we may distinguish various formed bodies. According to the type of cell there may be yolk platelets, pigment, fat globules, crystalline bodies, starch grains and many others. But there are three which are of sufficient general importance to merit the name of cell organs. The centrosome: a minute body, lying normally in close proximity to the nucleus and playing a remarkable part in cell division. The Golgi apparatus: an area of cytoplasm which can be picked out by suitable treatment with osmic acid, found in all nucleated animal cells except certain Protozoa, and regarding which recent researches show that it is undoubtedly associated with definite happenings in the cell. The mitochondria: minute granules and rodlets, intimately bound up with cell life and, like the Golgi apparatus, perhaps best summed up as the structural expression of only partly known cell activities.

Cell Division.—All organic growth proceeds by cell division. In the apparently simplest, but least understood, mode of division the cell elongates, narrows in the

middle like an hour-glass and separates into two, half the cytoplasm and half the nucleus going to each of the two resultant cells. This type of division is rare and its simplicity is perhaps only superficial.

The almost universal method is by mitotic division or *mitosis*, a wonderfully co-ordinated

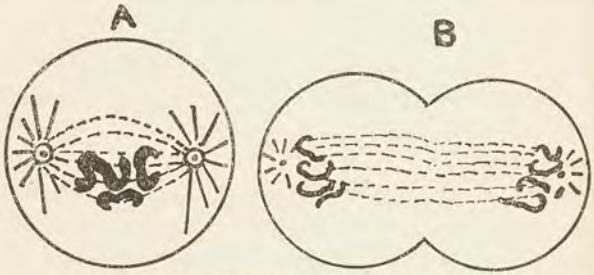


FIG. 26.—CELL DIVISION.
A. Earlier stage. B. Later stage.

series of events, of which we shall mention the chief.

The process centres round the nucleus and the centrosome. Just prior to division the chromatin of the nucleus forms into a long tangled thread, like a skein of wool. This skein then breaks up into a definite number of short stout rods (which are often bent) the *chromosomes*.

The centrosome divides into two and the two centrosomes recede to opposite poles of the cell. Between them there is an appearance of fine threads forming a " spindle " to the equator of which the chromosomes move. Each chromosome then divides *lengthwise* into two, and the two half chromosomes recede toward the poles of the spindle. When all the chromosomes have divided there is formed at each end of the spindle a group of half-chromosomes which mingle together as a mass of chromatin. The spindle disappears, the cell constricts and breaks and two new cells are formed, each with half the cytoplasm and half the chromatin of the original cell. Round the chromatin in each new cell a nucleus forms. Not only is there half the total chromatin passed to each daughter-cell, but half of each chromosome. In short, there is both a quantitative and a qualitative halving of nuclear substance.

At a time when the problems of reproduction and heredity were occupying the minds of the leading biologists of the day the appreciation of the importance of the nucleus in cell division had led to a rigorous investigation of the nuclei of the germ-cells and their behaviour in regard to the fertilisation of

the egg by the sperm. The gradual unfolding of such precise arrangements for a partition of the chromosomes in the dividing cell led in 1883 to one of the far-reaching discoveries in biology when Van Beneden demonstrated the central fact of fertilisation, that the chromosomes of the zygote are derived equally from mother egg-cell and father sperm-cell, so that every cell in the developing embryo contains an equal amount of nuclear material from both parents. Followed up by more recent researches, this discovery has had a profound bearing on the study of heredity and kindred problems. The number of chromosomes in the cell varies according to the species of creature. In general, it is constant for the species. Man has in each of the cells of his body 48 chromosomes : there are 40 in the pig, 18 in the hen, 26 in the bulldog, 32 in the bee; while among certain crustaceans and worms there are numbers like 8, 6, 4, and even 2.

Fertilisation of Egg by Sperm.—It will be asked at once how, if egg and sperm have a definite number of chromosomes, say 10, surely after fusion there will be 20 in the resultant zygote, twice the appropriate number?

At some period in the life-history of the

organism there occurs what is called meiotic division or meiosis : by which the cells destined to become eggs or sperms have the number of chromosomes halved; and hence when fertilisation takes place the specific number of chromosomes is restored. Meiotic division may occur early in life (though this is exceedingly rare), or in the middle of the life-history (plants), but in all body-forming animals and a few lower plants it occurs in the maturing germ-cells, usually in the last two divisions just before they are ready for fertilisation. These divisions are often spoken of as the maturation divisions. In the first of these divisions, lengthwise splitting of the chromosomes does not occur; instead, *whole* chromosomes are distributed to the daughter-cells, each of which thus gets half the full number. Normal (mitotic) division of these daughter-cells results in the formation of four cells, each with a half set of chromosomes. In the male all four are sperms, in the female one, the egg, is very large, the other three (" polar bodies ") very small. The polar bodies after being " thrown off " come to nothing.

Actual fertilisation takes place in higher animals in the body of the mother, in many

invertebrates in the surrounding water. The sperm touches the egg, from the periphery of which a little cone of cytoplasm often arises and encloses the sperm into the cytoplasm of the egg. Immediately the outer skin of cytoplasm separates off from the egg, forming an envelope —the fertilisation membrane—round it and preventing the entry of any further spermatozoa which might cause abnormal development. The sperm is so minute and contains so infinitestimal an amount of cytoplasm that little more than the nucleus can be seen after entry. A centrosome is, however, brought in.

The sperm nucleus moves toward the egg nucleus, which moves to meet it; the two come into intimate union, the full chromosome number is restored, and the zygote is complete. Immediately a spindle is formed and cell division sets in, the first step in the building of a new body; in some animals where the chromosomes can be clearly followed, it can readily be seen how during the early divisions of the embryo the quota of chromosomes to each cell is half-paternal and half-maternal in origin. As the new body grows by repeated division this precise distribution continues until finally the offspring arrives at maturity

with every cell in its body containing chromosomes from both parents. By careful studies on "cell lineage" it has been possible in a considerable number of animals not only to trace back the germ-cells of the adult to a definite cell or cells in the initial divisions of the zygote, but actually to delineate a particular area in the egg-cell as destined to give rise to the germ-cells of the embryo. There is thus not only an organic continuity between successive generations, in the sense that the fertilised egg-cell gives rise to a complete new individual, germ-cells and all, but a direct *germinal continuity* between parent and offspring. In other words, evidence points to the fact that very early, often at the outset, there is in the developing embryo a setting aside of the future germ-cells: they take no part in the building up of the general bodily organs, but remain segregated, housed in the body, until the time comes when they go to the starting of a further generation.

THE CHROMOSOMES AND HEREDITY.—The study of heredity is the study of the organic relation between successive generations. When we speak of some quality of body or mind as hereditary, and are using the term correctly, we mean that it is an *inborn*

quality handed on from an older generation to a newer, of whose *inheritance* it forms a part.

This inheritance is what the young organism starts out with in virtue of its hereditary relation to its forebears. Under appropriate conditions it is expressed or brought out in development, and when full growth is attained and the inheritance is realised we find that the young animal is very like its parents, though never *exactly* like them. In short, we notice that the qualities, physical and mental, of one generation somehow tend to reappear in the next. The question arises as to what is the physical basis for the transmission of such hereditary qualities? We have noted that there is a germinal continuity between one generation and the next, and it is clear that whatever is transmitted must be condensed into the compass of a single cell—the zygote—formed by the fusion of paternal sperm-cell and maternal egg-cell. Observation has shown that on the average the inherited qualities of the individual are derived equally from both parents. Consequently we have to look for something brought into the zygote in equal quantities by both egg and sperm. We find it in the chromosomes, an equal number of which are contributed, as we have seen, by

both parents. It is very tempting, then, to regard the chromosomes as the carriers of hereditary qualities. This must not be taken to mean the exclusion of other means of transmission hidden in the cytoplasm of the uniting parent cells, but working on this hypothesis a remarkable number of phenomena fit very well together. We have noticed the precision with which the chromosomes are divided in cell division, while the cytoplasm, as far as we can see, simply breaks, as it were, into two. The whole business of meiotic division in maturation seems to be for no purpose other than to ensure the right number of chromosomes in the mature germ-cell; so that when fertilisation occurs and the maternal and paternal shares of the inheritance mingle in the zygote, the normal arrangements of mitosis can distribute equal parts of maternal and paternal chromosome material to every cell throughout the entire body of the individual. It is generally accepted by biologists to-day that the chromosomes are the bearers of hereditary qualities, in part at least. The case is further strengthened by experiments similar to some we shall refer to later, where an egg bereft of its nucleus (*i.e.*, minus any maternal chromosomes) develops after union with a

sperm into an embryo showing only half the normal number of chromosomes in its cells and only paternal qualities or characteristics.

We may look further in regard to the significance of the chromosomes in heredity. The mode of inheritance which is called Mendelian, knowledge of which has been of such great value to the young science of Genetics, shows that very many organisms exhibit sharply defined " unit characters " which behave in inheritance in a very definite way. They do not blend in the offspring, but either appear in whole or not at all. Further, they are distributed in successive generations according to a definite scheme. Their representatives in the germ-cells are termed " factors." The conclusion was reached by inference from breeding experiments that the distribution of these well-defined characters according to the Mendelian law could only be accounted for by a sorting out or segregation of their factors in the germ-cells. Microscopical research has strikingly confirmed this. Professor T. H. Morgan and others have proved that these factors lie in the chromosomes; have shown in which chromosome particular factors reside, and have even indicated the exact position of the factor in the chromosome. It is in

the sorting out of the chromosomes in the maturation divisions of the germ-cell that the segregation of the factors for these unit characters takes place.

We may trace out the history of the factors for, say, blackness and whiteness in a case of two birds where black colour is " dominant " over white colour. Black crossed with white results in a brood of all-blacks. These bred together produce broods wherein there are " pure " blacks, " impure " blacks, and " pure " whites in the proportions 1 : 2 : 1. (The pure blacks and pure whites breed true, the impure blacks when bred have progeny in 1 : 2 : 1 proportion.) What is the cytological interpretation? In the original crossing the parents contributed severally a factor B for blackness and a factor W for whiteness to the zygotes whence their brood arose. Since B is dominant over W, W did not find expression in the inheritance and the brood were all black. W was there but latent. When this black-coloured brood grew up, then, their ripening germ-cells would contain factor B and factor W. Maturation of egg-cells sets in and in some of the eggs the separation of the polar bodies in meiosis carries out the chromosome containing B. In others the

chromosome containing W. Thus two sorts of eggs eventually mature, some with B, some with W. Similarly, maturation of the sperm-cells results in half the sperms containing the B-carrying chromosome and half the W. Breeding together takes place. There are four possible results.

W sperm + W egg gives W W in the zygote.
W ,, + B ,, gives WB ,,
B ,, + W ,, gives BW ,,
B ,, + B ,, gives BB ,,

Recalling that W and B are factors representing colours, the first and last will be pure whites and pure blacks, the chromosomes in their cells bearing only white and only black factors respectively; the middle two will be "impure" with both factors present, but since B is dominant over W, B only will be expressed in their colour, which will therefore be black. Thus we arrive at the Mendelian proportions 1 : 2 : 1, viz., 1 pure white, 2 impure black, 1 pure black.

A further illustration of the way in which segregation of chromosomes results in the presence or absence of a character is found in the cytological analysis of sex. In the egg-cells of a large number of animals there is one

more chromosome than in the sperm. Thus the female and male numbers respectively for the cockroach are 34 and 33, for the locust 30 and 29, for the dog 22 and 21, for the cat 36 and 35. In the cat, the mature egg-cell will have 18 chromosomes. In the case of the sperm, during the first meiotic division, one chromosome, termed the X chromosome, is observed to remain undivided, resulting in the ultimate production of two classes of sperms, one with and the other without the X chromosome, *i.e.*, with 18 and 17 chromosomes respectively. If one of the former fertilises an egg, the zygote becomes a female (18 + 18). If one of the latter a male (18 + 17). It is thus quite clear that the 18th male chromosome carries with it into the egg a factor which determines in some way that the zygote shall exhibit female sex and that in the absence of this factor male sex results. The X chromosome is thus known as the sex chromosome.

Variation.—Closely bound up with the handing on of established qualities from one generation to another is the question of the emergence of new ones. All plants and animals tend to vary, to change. The variations may be small, an extra petal to a flower, an additional feather in a wing, an extra plate

in a sea urchin's shell; or great, an Ancon sheep, an evening primrose, or a dwarf in the line of Anak. Variations are perpetuated by heredity. The origin of these variations, the origin of the distinctively new, is the most perplexing problem in biology to-day. Our ignorance is still profound, but perhaps not so profound as it used to be before cell study developed. We know now that in some way these changes originate in the germ-cells and are able to indicate opportunities at least for the emergence of certain kinds of variations. There is the phenomenon known technically as " crossing over " when during cell division in some animals pairs of chromosomes tend to twist round one another and even fuse temporarily, giving opportunity for rearrangements of chromosomal material—of " factors." Then during meiosis there is a throwing out of half the chromosomes from the egg in the formation of polar bodies. Thirdly, there is the intimate mingling of two sets of factors at fertilisation, when any marked alteration in factors would necessitate the attainment of a fresh equilibrium, and might result in the development of a new character. It is probable that many small quantitative variations arise in this way, but where qualitative

variations are concerned, complete new types, which involve a radical change of front on the part of the factors, or the emergence of entirely new ones, the mystery still remains.

Experimental Cell Study.—We may now turn to the experimental study of the structure and functioning of cells, and cite a few instances of an aspect of modern biology which is full of promise. The experimentalist may "experiment" with the outside of cells; may subject the whole cell or cells to various influences, chemical, physical or mechanical and study the results microscopically; or he may essay the infinitely more difficult task of penetrating into the cell itself under the highest powers of the microscope and by the most delicate manipulation work his will on its tiny contents.

A distinguished manipulator of cells is Professor Robert Chambers of Cornell University, whose investigations, particularly on cell membranes, have greatly furthered our knowledge of the physical properties of cells. The instrument designed by Chambers for micro-dissection, and which has been described by him in the *Journal of the Royal Microscopical Society* (1922) is an ingenious apparatus which may be attached to the stage

of the microscope, and which allows of the most delicate control of several micro pipettes or needles made of glass tubing drawn out to points invisible under the highest power objectives. The bore of the pipettes may be as small as $\frac{1}{25000}$ in. in diameter. The slide bearing the specimen is inverted over a moist

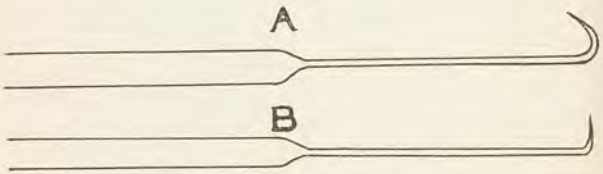


FIG. 27.—DELICATE GLASS NEEDLES MADE FROM FINE TUBING FOR MICRO-DISSECTION.

A. Needle with tip bent back for cutting purposes. B. Needle with tip bent up. For injection the extreme tip is tapped against the coverslip and broken off. A very fine pipette results. (After Chambers.)

chamber on the stage of the microscope; into this moist chamber the needles are introduced and the manipulation carried out under the oil-immersion lens. "The movements performed by the instrument are so accurately controlled that one can readily carry out such delicate operations as puncturing mammalian blood-corpuscles, tearing off the sarcolemma of a muscle-fibre, drawing out nuclear chromatin

strands and even cutting up the chromosomes of insect germ-cells. . . . With the micro pipette . . . one can either inject substances into or withdraw material from a cell" (Chambers, 1922). It places a great power in the hands of the experimental cytologist to be able to pull out loops of chromatin and remove chromosomes, as Chambers has done in the germ-cells of the grasshopper *Dissosteira*, or to determine directly the viscosity of different parts of the dividing cell during mitosis.

One of the great problems that modern cell study seeks to answer is, How is it that the qualities implicit in the fertilised egg are realised in the process of development; how does a complicated body of a definite kind grow from a single cell?

In most biological laboratories every year artificial fertilisation of the eggs of sea urchins and other animals is effected by allowing eggs and sperms to mingle in a jar of sea-water. The initial divisions of the zygote can then be studied and from time to time larvæ reared. It has been shown by Loeb and many others, however, that other things besides sperms will start the egg developing. In other words, artificial parthenogenesis can be induced in eggs kept clear of any influence of sperm;

the behaviour of the dividing egg started off in this way can then be studied in comparison with eggs fertilised by sperm in the normal way. Natural parthenogenesis where reproduction takes place without the intervention of a male occurs in a few animals, such as aphids and saw-flies among insects. Artificially it has been already induced in a number of types, starfish, sea urchins, worms, molluscs and frogs and in rare cases in plants, notably in the seaweed *Fucus*.

Various means are used and succeed in varying degrees with different animals. Many salts, carbon dioxide, and weak acids are efficacious added to the water in which the eggs are placed. Thus Loeb found that by adding magnesium chloride in certain proportion to the sea-water, sea urchin eggs could be induced to divide. After two hours of this they were replaced in sea-water and eventually developed into larvæ. Increasing the density of the surrounding medium, alterations of temperature, electrical stimuli and even shaking may start off the ripe egg dividing. Perhaps most curious of all methods is that of Bataillon, who punctured frogs' eggs with a very fine needle which had been dipped in frog blood or lymph. Though successful, it

is perhaps right to add that the proportion of fatherless tadpoles resulting was not very great.

A further line of experiment involves interference with the normal course of development during the early divisions of the zygote. Destroying or pressing one of the first two cells, shaking the cells apart and observing the effect. If the dividing fertilised eggs of many marine animals are placed in sea-water minus its tiny quota of calcium chloride, the cells cease contact with one another, and a mass of dissociated living cells results.

A zygote divides into 2 then 4, 8, 16 cells and so on. In the sea urchin if dissociation of cells of the 2-, 4- and sometimes 8-cell stage be effected, each isolated cell will develop into a minute dwarf larva. The same holds good for the lancelet. In the newt a fine loop of hair tied tightly round the junction of the cells in the 2-cell stage results eventually in the development of " Siamese twins." A less tightly tied loop produces a two-headed animal. Professor E. B. Wilson showed that if a certain part of the protoplasm (the polar lobe) is removed when division has started in the fertilised egg of the mollusc *Dentalium*, the resultant larvæ are markedly abnormal

and finally die off. Even before fertilisation if the egg is cut into two with a fine scalpel *through a certain plane*, and both halves are fertilised, one will become a normal though dwarf larva, the other a thoroughly abnormal one. Such experiments indicate the subtlety of development especially in regard to the distribution or localisation of the stuffs in the dividing egg which are destined to give rise to particular organs.

We have seen in the preceding chapter that after the blastula and gastrula stages in the developing embryo three primary layers of cells are formed and differentiation soon sets in, the rudiments of bodily organs making their appearance. How is this differentiation induced? Professor Spemann has recently made a notable contribution toward the understanding of the process. Working on the development of the newt, he came to the conclusion that a particular area in the embryo (the dorsal lip of the blastopore) was closely associated with the onset of differentiation. By skilful micro-surgery he excised a part of this area and grafted it on to a second embryo, with the result that a supernumerary set of embryonic organs formed under its influence. Repetition of the experiment under conditions

where distinctive pigmentation made it possible to distinguish clearly the grafted area in its new surroundings made it clear that the graft actually was a differentiator, that it took little or no part in the building of the extra embryo, which was formed from the host under the influence of the graft.

Of recent years much progress has been made in the cultivation of living tissues outside or excised from the developing embryo or even adult body. Fragments of tissue are removed under strictly aseptic conditions and placed in a drop of nutrient plasma or lymph on a coverslip. The coverslip is then inverted over a hollowed microscope slide and the rim sealed. In such media the growth of the tissue steadily continues. Early embryonic tissues especially will live for weeks, sometimes years with care. The same specimen can be studied continuously, and changes of form and position and division of cells watched under the microscope. Thus Professor R. G. Harrison, wishing to test the powers of embryonic cells to form nerve-fibres by outgrowth, dissected out bits of the spinal cord of frog and toad tadpoles and studied them in clotted frog lymph. He found that the nerve-fibre was formed by an outflowing of the cytoplasm of

the young neurone, nerve-fibrils being afterwards differentiated within the slender process. He was able to measure the rate of growth and found that the fibres grew from $\frac{1}{1600}$ in. to $\frac{1}{450}$ in. per hour. The greatest growth made by any one fibre was rather less than $\frac{1}{20}$ in. over a space of two days. His observations demonstrated quite clearly the way in which the primary nerve-paths of the body are established by protoplasmic out-stretching of the nerve-cells.

To conclude, it is not possible within our present limits to take the reader far along the fascinating way of the higher microscopy, where the biologist finds himself at close quarters with life itself. We have done no more than give glimpses of the phenomena which come within the ken of the student of cells, and hint at some of the problems on which he is engaged. It would be idle to deny that cell study is difficult, for the subtleties are extraordinary. It demands the best microscopical equipment and the best brains behind it; but it is one of the most promiseful developments of modern biology.

CHAPTER VIII

A HISTORICAL CHAPTER

DELIBERATELY ignoring the vexed but unimportant question of who invented the first microscope, we may now pass in review some of the achievements in the domain of microscopy in the not very remote past. And it will be surprising if we do not feel a touch of wonder at the earnestness of those early microscopists and at the results they achieved with their feeble instruments. They did so much with so little. It is desirable to point out that the term microscope as we use it refers to what was originally called the " compound microscope " in contradistinction to the early " simple microscope " nowadays represented by the hand lens or pocket lens. These simple microscopes of the earlier worker were mere globules of glass, frequently made by the microscopist himself by melting fragments of Venetian glass in a flame and thereafter cementing in a tiny hole in a metal plate.

Gradually improved by discoveries in lens construction, the simple microscope was the favourite instrument of workers for a very long period. Not till the nineteenth century was entered on did it give way to the by that time much improved compound instrument. The compound pattern had been long known and used, but had many drawbacks. Hooke's pattern dates from 1665. Redi's *De Insectis* figures a primitive type over the date 1670, and there are a host of succeeding styles, many remarkable as examples of the silversmith's art and for their decorative design rather than their optical efficiency. The difficulty lay in the fact that the old-time lenses were uncorrected, and while with single lenses distortion was minimised by using only the central part of the field of view, the use of two such lenses, one over the other, in the compound instrument led to long unsolved optical problems, so that even at the beginning of last century one able microscopist declared that the compound microscope would never beat the then improved simple pattern. Even Darwin, it is said, took no compound microscope with him on his famous "Beagle" voyage, but only a simple one. However, the introduction of achromatic microscope

objectives in the period 1820–30, revolutionised microscope construction and from that time there has developed the very perfect instrument we know to-day.

The seventeenth century was a time of restlessness—intellectual restlessness. The first fruits of the revival of learning had already shown themselves in various ways, chief of which was a widespread keen desire to know more of the world of Nature and the nature of things. Ray was laying the first stones in the foundation of modern natural history; Harvey had proved the circulation of the blood in 1628; the Royal Society of London had been incorporated in 1662. It was an era of achievement in observation and experiment.

First Period.—From about the middle of the seventeenth to the middle of the eighteenth century. The coming into use of a means of making small things loom large had the same fascination for the early microscopists as a telescope has for a small boy. It was trained on everything they could think of. Filled as some of them were with an appreciation of its value as an aid in purposeful inquiry, yet the field was so vast, the wonders met with at every turn were so many, that it

is not surprising that the first or early period of microscopy was in the main a period of observation—of the amassing of countless new facts. At work in a hitherto inaccessible field, fascinated with their new eyes, the pioneers of microscopy ranged over the whole of zoology, botany, physiology and anatomy, here and there chronicling discoveries, many of which stand out as landmarks in the history of science. They went about their work in a spirit well indicated by the words of Malpighi: "In performing these researches, so many marvels of nature were spread before my eyes that I experienced an internal pleasure that my pen could not describe."

Typical of these men was Antonius à Leeuwenhoek (1632–1723) Sheriff's Chamberlain of his native town of Delft (at a salary of twenty-six pounds a year). He made all his own lenses—tiny double convex lenses mounted between two plates of silver perforated with a small hole with a movable pin in front of it on which objects might be placed. He was a man of wide interests and the variety of his microscopic observations is astounding. His letters to the Royal Society of London, of which he was a member, are famous. In the course of a long and vigorous life, he wrote

nearly four hundred of them. It is plain that he followed no systematised plan of study, but turned his microscopes this way and that. So keen an observer as Leeuwenhoek was bound to make discoveries, and the list is a long one. He was the first to observe bacteria, protozoa, wheel animalcules, the capillary circulation in tails of tadpoles and fishes, and to distinguish the oval shape of the blood-corpuscles of fishes, birds and frogs from the circular ones of mammals. He showed the branching in heart-muscle, the banding of striped muscle, the little "boxes" in plant tissue, noted also by Hooke, which turned out to be cells. He also published the first descriptions and figures of "seminal animalcules" (spermatozoa) after his attention had been drawn to them by a Leyden medical student. Nothing came amiss to him, animal, vegetable or mineral. Many of his letters deal with the crystals of common substances. He noticed that, when the seminal fluid of a fish was diluted with (probably fresh) water, the spermatozoa burst into pieces, an observation which is interesting in view of recent experimental work in cell-physics. Occasionally Leeuwenhoek allowed himself to be carried away by his imagination, when he discerned

muscles and other organs in animalcules, or a complete lamb in a three days' sheep embryo; but he retained sufficient native caution to demand (and obtain) from the Fellows of the Royal Society an affidavit that they had actually seen in a drop of water the animalcules whose existence he had demonstrated to them. The minuteness of so many living things led him into hosts of calculations, a common habit among the earlier microscopists. Some of these efforts are highly amusing to the modern reader. The following will serve as an example. On examining the spermatozoa of the cod, Leeuwenhoek *estimated* that it would take ten thousand of them to equal the bulk of a grain of sand. One hundred grains of sand go to the inch, therefore a million to the cubic inch. The reproductive organs of the cod are of 15 cubic inches bulk, equal to fifteen millions of sand grains, totalling 150 thousand millions of sperms. He goes on to calculate out the surface of the earth at 9,276,218 Dutch square miles. Estimating one-third to be dry land, and allowing that two-thirds of this is inhabited, and that Holland and West Friesland between them total 154 square miles, then the habitable part of the world is 13,385 times the size of Holland

and West Friesland. Further, if the population of these provinces is a million, and if all other parts of the world were as populous as

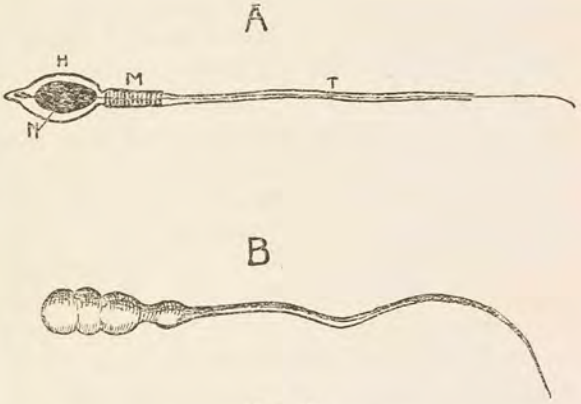


FIG. 28.

A. Structure of a spermatozoon: H. head; M. mid piece; T. tail; N. nucleus. B. Leeuwenhoek's drawing of the spermatozoon of a dog (from Leeuwenhoek, 1687).

they (which, he naïvely adds, is highly improbable), then there are 13,385 million people on the earth. All this to reach the conclusion that a male codfish contains spermatozoa ten times more numerous than the inhabitants of the globe: "*plus decies superare homines in terrarum orbe viventes.*"

A more scientific and not less keen observer was Marcello Malpighi (1628–1694), sometime professor of medicine at Bologna. His studies of glands and tissues form one of the foundations of physiology and in investigations on the blood he showed himself a worthy follower of Harvey. He described the minute structure of the lung in the frog and other animals, and saw the circulation through its capillary vessels. His name remains to this day in the Malpighian layer of the skin and the Malpighian bodies of the kidney.

In a great work on plants with 93 plates of figures, Malpighi, together with Grew (both works were presented to the Royal Society on the same day) laid the foundations of the microscopic study of plant structure. He noticed the cells of plants, which he called "utricles." As an observer of development, Malpighi merits a high place. His description of the development of the chick illustrated by well-drawn figures is far in advance of many of his successors and a model of painstaking and, considering his instruments, remarkably accurate observation. Malpighi made another step. He turned to the structure of invertebrate animals, and his famous monograph on the silkworm was the opening of a new

field of study. Breathing tubes, nerve cord, food canal, silk glands, all were dissected and drawn, in spite of persistent eye trouble induced by the exacting work. The kidney tubes of insects are still called Malpighian tubules.

The fame of Malpighi lies in the number of lines of investigation he started, utilising to the full the newly introduced microscope; plant structure, animal structure, development. To his keen mind, too, is owed the beginning of the comparative study of structure, by his employment of the fruitful method of seeking resemblances between different types of animal.

Far less of scientific mind than Malpighi, but almost unparalleled in the intensity of his study and the deftness of his manipulation, the third member of the illustrious trio who, by common consent, stand out among the early microscopists, was Jan Swammerdam, of Amsterdam. Born in 1637, by the age of thirty-six he had exhausted his powers, and after a few years of religious mania died in 1680. It is clear that he was nearly, if not quite, crazy, and his craze took the form of an intense application to manipulative work. At this he was superb. Insect structure was

his chief study. His dissections of insects, aided by the microscope, were extraordinary, his observations exact to a degree, his drawings exquisite. Eye trouble followed his hour-long observations with simple microscopes in full sunlight, but he worked on; observing by day, describing and drawing by night. The pace was too hot to last, and eventually he broke down. In his "Biblia Naturæ" are the results of his application: marvellous results for the time (and even for any time) considering the lenses available. Swammerdam worked through the life-history of the bee and many other insects, on the structure of the snail and frog, and various other types, dissecting, describing, drawing with extraordinary skill. Part of his success was due to his ability to grind fine scissors, scalpels, etc., suitable for microscopic dissection and to fashion delicate pipettes for injecting minute organs. It seems as though Swammerdam never had time to think over what he laid bare, for his work lacks the philosophic insight of Malpighi, but he stands out in the history of microscopy as the masterly observer.

One of the early books on microscopy published toward the close of the early period gives a fair idea both of the state of scientific

thought at the time, and the wide range of the microscopists of the day. In view of the uncertainty surrounding the exact date of birth of microscopy, it is interesting to notice that the writer (1742), states: "It is something more than a hundred and twenty years since the Microscope was happily invented; and to the valuable discoveries made thereby, we stand indebted . . . for a great part of our present Philosophy." More might have been achieved by it, but "At the beginning, it was confined to very few; who, making a secret of it, endeavoured all they could to keep it to themselves; and when it became a little more publick, the Price was fixt so high, that the most Curious and Industrious, who have not always the greatest Share of Money, could not conveniently get at it." Our author was both curious and industrious, for he deals, amongst many other subjects, with animalcules, blood, muscle fibres, nerves, bone, the skin, lice, mites, fleas, spiders, gnats and other winged insects, snake poison, the "balancers" of flies, fish scales, oysters, seeds, leaves, crystals of various salts: under Miscellaneous Discoveries come parasitic worms (he mentions the hooks of the tapeworm and had ideas on its life-history); liver flukes ("like a small,

thin Myrtle-Leaf with a very short Foot-stalk "), aphids and ants, crane flies " called by the common People *Father-long-Legs* " ; mosses, pith, sand, snow flakes, and seeds.

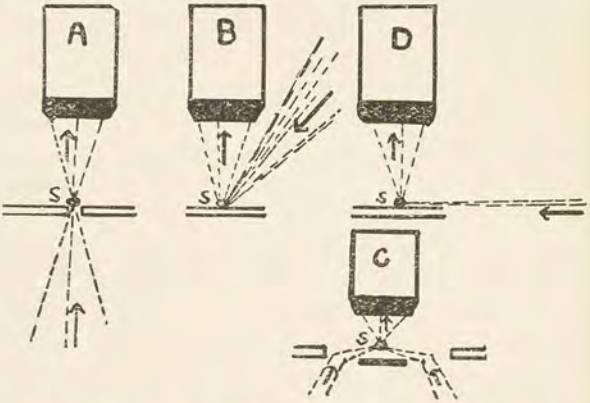


FIG. 29.—METHODS OF ILLUMINATING THE SPECIMEN.

S. Specimen. A. Transmitted light. B. Reflected light. C. Very oblique light (dark ground illumination). D. Horizontal lighting (ultra-microscope).

" Kind Nature has supplied the Seeds of Dandelion, Thistles, and many other Plants with a *Down* that serves instead of Wings to convey them to distant Places. The Figures of such *Down*, in different Plants, are very different when look'd at through Glasses :

some appearing plain and smooth, others rough and thorny, and others again with little Hooks or Claspers to catch hold of anything" (Baker, 1742).

SECOND PERIOD.—FROM ABOUT THE MIDDLE TO THE CLOSE OF THE EIGHTEENTH CENTURY. The work of the earlier observers, who too often hardly understood what they saw and drew, opened the way to deliberate studies on various forms of plant and animal life at a deeper level than before, aided by improvements in lenses. Our second period is marked by an advance in the standard of critical observation rather than progress in the fuller interpretation of what was seen. It is noteworthy in respect of several famous monographs which showed a setting to work on systematic lines, and for the prominence of several questions of general interest in which microscopists of the day shared.

In 1744, Trembley published his famous monograph on the freshwater *Hydra*, a notable contribution to biology, illustrated by beautiful plates engraved by Lyonet. In addition to a very thorough study of the general natural history of *Hydra*, Trembley instituted experiments which disclosed the remarkable powers of regeneration possessed

by many simple animals. On cutting off the head of the tiny double-walled tube which forms the body of *Hydra*, a new head grew; cutting lengthwise, the parts closed up and two individuals resulted; cutting into four produced four complete *Hydras*. He even found means of turning the creature inside out as one may turn a stocking, and it adjusted itself to the change. The matter greatly interested Reaumur (of thermometer fame) a keen microscopist and observer, who, with others, sought and found evidence of regeneration in other animals, notably various worms and starfishes.

A descriptive monograph of a remarkable nature was brought out in 1750 by Lyonet on the anatomy of the caterpillar of the Willow Moth. In Lyonet, skilful dissection under the microscope and brilliant draughtsmanship reach a climax. He drew all his figures, 137 of them, on copper, and the detail is amazing. He distinguished and drew over 4,000 muscles in the Willow Moth caterpillar and his studies on the other systems of organs were equally detailed. The head of the caterpillar is little larger than the nail of a man's little finger, but Lyonet made a series of seven dissections showing the whole of the structure of the head :

muscles, nerves, ganglia, air tubes all traced to their terminations. The drawings of these dissections are among the most remarkable plates ever figured.

The eighteenth century witnessed much of the arguments concerning the possibility of spontaneous generation, which were not settled till the days of Pasteur, and most of the prominent microscopists took sides in this controversy. But of greater interest, as a chapter in the history of Embryology, were the hot discussions that arose concerning the nature of development. The technique of the day could not reveal the really early stages of development; the apparently sudden appearance of certain formed parts in the hitherto simple egg, perhaps coupled with the erroneous idea that the animalcules (sperms) which entered the egg had a miniature equipment of organs similar to large animals, had led to the idea that the fertilised egg contained a fully formed minute animal, which expanded or unfolded itself in development, as an embryo plantlet in a seed. The adherents of this view, which was supported by much metaphysical argument were called "preformationists." They were divided among themselves as to whether it was the

egg or sperm that contained the miniature. Logically followed out, the preformationist doctrine involved the encasement in any one embryo of all succeeding generations. A contemporary wrote :—

" Each seed includes a Plant : that Plant, again,
Has other Seeds which other Plants contain :
Those other Plants have all their Seeds ; and, those,
More Plants, again, successively, inclose.
Thus, ev'ry single Berry that we find
Has, really, in itself whole Forests of its Kind.
Empire and Wealth one Acorn may dispense,
By Fleets to sail a thousand Ages hence :
Each Myrtle-Seed includes a thousand Groves,
Where future Bards may warble forth their Loves,
So Adam's Loins contain'd his large Posterity,
All People that have been, and all that e'er shall be.

Amazing Thought ! what Mortal can conceive

Such wond'rous Smallness!—Yet we must believe
What Reason tells: for Reason's piercing Eye
Discerns those Truths our Senses can't descry."

Against this speculation run mad Caspar Wolff in 1759 raised a protesting voice. He had studied the formation of some of the organs in the chick and in various plants, and appealing to facts pointed out that these organs made their appearance gradually and were to be seen being formed. He averred that development was a gradual process of becoming, rather than the expansion of a preformed miniature. But the preformationists were too strong for him. Wolff was, in fact, howled down. The leading men of the day, including Haller, the distinguished physiologist, were nearly all in the opposite camp; and the study of development was stultified till the following century was well under way, and Pander and Von Baer, working with improved instruments, unfolded the true nature of the process and vindicated Wolff.

A distinct feature of the latter part of the

eighteenth century was the progress made in knowledge of the simplest animals and plants. From the time of Leeuwenhoek, the " world of invisible life " had been known but its inhabitants had been largely regarded as wonders of nature—microscopic curiosities. Perhaps their extraordinary variety and amazing numbers discouraged systematic study. Did not Linnæus—so precise and orderly—group them as " Chaos " ? O. F. Müller made a determined attempt to set things in order and his treatise on Infusion Animalcules, published in 1786, was a foundation work. He tried to classify them on the Linnæan principle, and though subsequent studies have made great changes in his classifications, he remains a notable figure, one to whom present-day students of the microscopic side of pond life will pay homage.

By the end of the eighteenth century the foundations of microscopy had been laid. With the exception of a few observations like those of Reaumur on iron and the beginning of the use of the microscope by chemists for seeing minute crystals, nearly all the work had been of a biological nature. Masses of facts about the minute structure of animals and plants had been accumulated, soon to be

tested in the light of tissue knowledge and the cell theory. Notable aid had been given to the then flourishing study of comparative anatomy, and to physiology. Microscopy was established as a useful handmaid of science.

THIRD PERIOD.—NINETEENTH CENTURY. The rising tide of microscopy flowed into the new century, gathered way and swept on rapidly and wide.

From this time onwards the development of microscopy, technically and in its applications, is a marked feature in the history of science. The compound microscope, later to be improved out of all recognition, gradually supplanted the simple pattern. The various fields of microscopical study were marked out. The microscopy of biology began to diverge along three main lines: the study of minute structure, of minute organisms and of development. Later on geological microscopy took shape, then metallography. It is out of the question to attempt a detailed history of a period big with discovery; but we shall trace the progress in outline.

There were profound achievements in microscope construction. The phenomena of polarised light were under consideration by

physicists and Brewster applied polarised light to the simple microscope, thus paving the way for Fox Talbot, who in 1834 fitted nicol prisms to the compound microscope. The introduction in England and on the Continent of achromatic microscope objectives was a very big step. Of equal importance was the recognition of the necessity for optical perfection in the condensing lens below the microscope stage.

On the threshold of the new century stands Bichât, whose early death in 1801 cut short a brilliant career. Bichât was not a microscope user, but his influence on microscopy was so stimulating that he is commonly referred to as the "father of histology." He took the definite step of passing from the study of organs to the study of their constituent materials, tissues, as a means of insight into the animal body. "The anatomist had disclosed organs such as heart and lungs : Bichât analysed these organs into their component tissues (muscular, connective, nervous, etc.), and also endeavoured to show that the function of the organ was expressible in terms of the properties of these tissues" (Thomson).

A fresh phase of microscopy was thus entered on, but before the new study reached fruition

the cell theory was announced. In its light, histology took on a new meaning.

It is interesting to notice the conditions under which the cell theory was set on foot. The work of Bichât and a reviving interest in development had busied investigators, botanists and zoologists alike, with the nature of minute structure. From the "Micrographia" of Hooke onwards, numbers of the drawings of the early microscopists figured the cellular structure of plants, *e.g.*, the "utricles" of Malpighi—but there had been no appreciation of the cell as the basis of plant structure. Animal bodies were built of "globules" (Wolff). Haller roundly declared all organisms, plant or animal, to be made up of "fibres." Gradually among botanists, especially, the term cell crept into use; for it is a very natural name for the thick-walled "little boxes" of plant tissue. In 1831 Robert Brown discovered the nucleus of plant-cells. He grasped the idea that the nucleus played an important part in the formation of new cells, but drew erroneous conclusions as to the nature of the process, conclusions which were not amended for some time after the cell theory had been launched.

The two microscopists whose names are

associated with this epoch-making generalisation were Schleiden and Schwann, the former a botanist, the latter a student of both plant and animal tissues. Schleiden published his work in 1838, and showed that plant tissues are built up of cells, and that the plant begins as a single cell. Schwann, in touch with Schleiden's work, found therein confirmation of his own observations on animal tissues, announced his conclusions and published them in 1839. From that year dates a new era in the study of organic structure. It was laid down (1) that all plants and animals are either single cells or built up of cells; (2) that plants and animals, reproduced in the ordinary way, begin life as single cells, from which in other than unicellular creatures a body is built up by cell formation; (3) that the functioning of this body is bound up with the functioning of the cells which compose it.

" It may be asserted," says Schwann, " that there is one universal principle of development for the elementary parts of organisms, however different, and that this principle is the formation of cells."

This far-reaching conception of a fundamental unity in all living things coloured all biological microscopy from 1840 onwards.

We may follow out its effect on cell study first. There was concentration on the nature of the cell substance. Careful comparison and research began on the resemblances between the jelly-like ground mass found in plant-cells and animal-cells. Gradually it dawned that the "sarcode" of animals and the "schleim" of plants were one and the same, and eventually, in 1861, Schultze brought the matter to a head, and extended the cell theory by showing that all cells, plant or animal, are made of the same fundamental living stuff—protoplasm.

The nature of cell formation took a considerable time to find out. By 1858 Virchow was able to state definitely that every cell arises from a pre-existing cell; "*omnis cellula e cellula.*" It was found that both the nucleus and the cytoplasm each divided in the formation of new cells. The complicated and orderly process of mitotic division was not, however, made clear until after the introduction of methods for fixing and staining. By 1870, or thereabouts, the ground had been cleared, and modern cytology begins, soon to make important contributions towards the understanding of heredity.

The cell theory raised new questions in

regard to minute organisms. Following O. F. Müller, the systematic investigation of the smaller forms of life was carried on by Ehrenberg. His great researches published in 1838 are a marvel of patient microscopy. Ehrenberg distinguished as a separate group the wheel-animalcules or Rotifers, which Leeuwenhoek had discovered, and his work in this direction stands to-day. He had, however, a curious misconception regarding the organisms which constituted his group " Polygastrica "—many stomached. In this group were protozoa, bacteria (he was the first to use the name), diatoms and other minutiæ. Misled by the fact that certain protozoa showed in their unicellular bodies several vacuoles filled with carmine particles he had fed to them, Ehrenberg was led to attribute to his " Polygastrica " a far higher grade of internal organisation than they possess. Considering that he was equipped with decidedly better appliances, it seems strange that Ehrenberg should have returned to something very like the old view of the pioneer microscopists. But it must be remembered that even yet the happenings inside the minute creatures amongst which he laboured so ardently are far from being understood. How-

ever, strangely enough, his work was hardly published when the newly arrived cell theory introduced a quite different conception of unicellular organisms. Ehrenberg stood his ground for a time, but the result was inevitable, and in the classification of Von Siebold in 1845 " Polygastrica " dropped out and " Protozoa " came in to stay.

To return to the study of development: the old doctrine of preformation faded away in the light of new knowledge. Pander (1817) made a step in his study of the chick; but his fellow-student Von Baer, who in 1828 discovered the egg of mammals, brought a wide range of new types under his microscope. From his comparative studies, he grasped the nature of development as no one had done before. He holds the honoured title of the " founder of modern embryology." The first divisions of the zygote of the frog were noted (but not fully understood) in 1826. Von Baer with masterly insight traced out the formation of tissues, and from the formed tissues the building of organs. Thereafter, Remak (1855) and Von Koelliker interpreted development in terms of the cell theory and the modern study of animal development may be said to have begun.

Plant development followed closely. Brown and Schleiden took the lead on the botanical side, but a little later Hofmeister out-distanced all his fellows and did for plants what Von Baer had done for animals : he broadened the study of development and placed it on a comparative basis. His chief work was on ferns, mosses and flowering plants; and he is well known as the discoverer of the alternation of generations in plants.

The early years of the nineteenth century afford interesting foreshadowings of the widening application of microscopy. Widmanstätten examined polished specimens of meteoric iron in 1808. In 1832, François followed under the microscope the reduction of iron from the ore. The microscope began to be used to examine the minute external configuration of rocks. Brewster in 1814 studied a large number of miscellaneous substances under the polarising microscope, "hairs and bristles, cuticles and corns, glue, isinglass, horn, paper, gums and balsams, waxes and organic acids." It was not, however, till later in the century that the big steps were taken in the microscopy of rocks and of metals, both of them associated with the name of Sorby.

We cannot close this brief survey of the microscopy of yesterday without reference to the genus "microscopist." We mean those enthusiasts, sometimes men of science, often not, who have done so much for the improvement of the microscope and the spread of a fascinating recreation. It is safe to say that most of the improvements in microscopic equipment in the past have emanated from the now less common "amateur" microscopist. In a less sophisticated age, too, the popularising of a modest form of microscope did much to arouse a healthy curiosity regarding the nature of living things and stimulate an interest in scientific inquiry.

CHAPTER IX

THE PROGRESS OF MICROSCOPY

In bringing to an end this short survey of microscopy, it is natural to ask What are the prospects of further achievements through the employment of the microscope which has brought so much within man's ken? It is a question not admitting of ready answer. It might be said that a still greater army of workers in those branches of science where the microscope is employed would lead to new and perhaps great results. There is much to be said for this. Or perhaps a careful looking round for new fields of work would bring fresh triumphs. This is undoubtedly true in the realms of industry. In industrial microscopy the foundations are hardly yet laid, and though progress must be slow for a while, there is a bright future.

Again, there is room for great additions to our present knowledge of the microscopical side of heredity and cell life and of disease-

causing organisms. There is no dearth of problems to be solved.

On the other hand, there is a definite limit of vision, theoretically calculable, beyond which the ordinary microscope as at present constructed cannot reveal things as they are, and we wish to make this clear even though it involves technical details. We must distinguish between merely seeing an object, and seeing the structure of an object. That is, we must distinguish between *visibility* and *resolution*. It has already been shown how, by means of the "ultra-microscope," the presence of excessively minute bodies can be detected under certain conditions, visibility being theoretically limited only by the ability of the particles to reflect sufficient light to show their whereabouts.

But this does not show their structure. What is the limit of resolution, *i.e.*, of the power of separating out fine details? We have previously stated that the index of resolution of an objective is its Numerical Aperture. Now, when we approach the limits of resolution, the nature of the light used has also to be taken into account. The structure of an object smaller than half the wave-length of the light which illuminates it cannot be

seen owing to diffraction. Looking at the tables of Aperture and Resolution in the *Journal of the Royal Microscopical Society*, we find that the best objectives of N.A. 1·40 have a limit of resolving power of slightly under 135,000 lines to the inch *in white light*. This means that the most that such an objective is capable of doing is to show, as two separate lines, a pair of lines in an object which are $\frac{1}{135000}$ in. apart. But we find that the same objective will resolve 146,000 lines to the inch in *blue light*. Now the wave-length used in observing by white light (line E) is 0·5629μ ($\mu = \frac{1}{1000}$ of a millimetre) and that of blue light (line F) is 0·4861μ. So that by using light of shorter wave-length a certain increase of resolving power may be obtained.

A further step is possible. The human eye is not very sensitive to the blue-violet region of the spectrum, and it is difficult to work with light of such a colour. But a photographic plate is far more sensitive than the eye to blue and violet light, and by using a plate very sensitive to violet further resolution is possible. Continuing our reading of the above-mentioned table, 177,000 lines to the inch may be photographed with light of 0·4000μ (near line h).

The question then arises, Can light of yet

shorter wave-length not be used? Visible light ends about wave-length 0·3600μ. Below this comes the ultra-violet, unseen by the eye, but very potent in its effects, especially on bacteria and living tissue generally.

Ultra-Violet Light.—Since the ultra-violet rays are invisible, observation with the eye is out of the question and it is necessary to rely on photographic plates sensitive to ultra violet. The rays can, however, be detected, and the image they form seen by allowing them to impinge on a specially prepared screen which is made to fluoresce where the rays strike it. The resolution obtainable is theoretically nearly twice what is possible using white light, but it must be confessed that the formidable nature of the operations and the very great cost of apparatus, together with the restrictedness of its applications, have stood in the way of any widespread employment of ultra-violet light for microscopic work.

Mr. J. E. Barnard, F.R.S., writes: "All bodies to be photographed by this means must be unstained, and any detail of structure will be brought out as the result of differences in the absorptive-power of the tissue to ultra-violet light. The preparations must not be fixed, dried or otherwise treated in the way

that microscopical preparations are usually dealt with. It is also important to remember that the medium in which the preparation is immersed must be one that does not offer any obstruction to the passage of ultra-violet light."

The light is obtained from an electric discharge between magnesium or cadmium electrodes and is usually of wave-length 0·2800μ or 0·2750μ. Owing to the opacity of glass to ultra-violet rays, the whole of the glass parts of the microscope must be made of *quartz:* condenser, slide, coverslip, objective and eyepiece. A mixture of glycerine and distilled water replaces oil as an immersion fluid for the objective. Canada balsam, being opaque to ultra-violet, cannot be used as a mounting medium and glycerine or agar-salt solution is used instead. A great difficulty in using ultra-violet light is focussing with the fluorescing screen. The notable investigations of Mr. Barnard into the nature of filter-passing microbes have been facilitated by certain improvements in apparatus, especially by the construction of a new pattern of condenser, used on a microscope the stand of which is of special design to give great stability and exceptional accuracy in all movements. The

condenser is, in a way, two condensers in one, ingeniously combined. Using visible light, green in colour, the specimen is found and focussed under oblique (*i.e.*, dark-ground) illumination. Thereafter a beam of invisible ultra-violet rays is passed through the hitherto unused central part of the condenser and pictures the specimen on a photographic plate. In addition to accurate focussing this allows the ultra-violet light, which is extremely injurious to living organisms to be withheld save while the actual exposure is being made. The resolution of details of structure obtainable by dark-ground illumination is sufficient to resolve perfectly details of a specimen $0{\cdot}35\mu$ in diameter, but with ultra-violet light a photograph was obtained "that under favourable conditions would give an image of an isolated element of structure down to an order of size of $0{\cdot}075\mu$."

X-Rays.—In photographing by ultra-violet light the shortest wave-length hitherto used is $0{\cdot}2570\mu$. Far shorter in wave-length than ultra-violet light are *X*-rays, and it may be that they will yet be turned to good account in microscopy. Mr. Barnard, who has done so much to explore the possibilities of microscopy, has given an interesting account of

his experiments with *X*-rays of a wave-length a thousand times shorter than the shortest of ultra-violet rays : and we follow his account. Some years ago, the method was tried of observing through the microscope the image of a minute object formed on a fluorescent screen by Röntgen rays. Lack of brightness and the granularity of the screen made this procedure of little value. Mr. Barnard tried another method. " An *X*-ray tube is enclosed in a lead-covered box so that the rays cannot pass out except in the desired direction. There is a small aperture in the position occupied by the lithium glass window of the tube, and this is covered with a lead diaphragm which has a small central circular hole. There are two or three more of these diaphragms, one above the other, with a certain distance between, so that at a distance of 15 to 20 centimetres from the tube a parallel beam of *X*-rays of very small cross-section is transmitted. The object is then laid in a light-tight box, in the path of the beam, the rays passing through the object and impinging on to a photographic plate which is placed in actual contact with the object. The photograph then results as in ordinary *X*-ray work."

It will be observed that the microscope does not enter into the operation at all. What is achieved is a minute *X*-ray negative of the specimen, which in the case referred to included various foraminifera; the tiny negative is then enlarged in the usual photographic way. The difficulty in using *X*-rays in microscopy lies in the fact that they do not behave as ordinary light rays do in passing through the optical system of the microscope. "The problem to be solved at the present time is to find a method of expanding or contracting a beam of *X*-rays in the manner that a lens alters the form of the wave front with ordinary light" (Barnard, 1915).

We see then that for everyday practical purposes, it is as yet too early to hope for increase of resolution much beyond present limits and there is plenty of scope for valuable work within those limits if a sound knowledge of the manipulation of the microscope accompanies it.

The close relation between microscopy and photography will have become apparent to the reader by the foregoing paragraphs. Photography serves two ends in microscopical investigation. One, as we have seen, is to register an image formed by invisible light

which the human eye cannot perceive directly; the other is to record what is seen in ordinary microscopical work. There are other means of achieving this latter purpose. The microscope picture may be drawn from observation by a skilful draughtsman, helped maybe by various aids; or the image may be projected on to a paper-covered board and the details of the specimen traced out. But photography, or photomicrography, to give it its proper name, has several advantages. It is rapid, at least in skilled hands; it is accurate, preserving spatial relations perfectly. A single good negative will yield unlimited prints whereon, if need be, the desired structures can be outlined in waterproof ink and the unwanted silver image bleached away. The chief disadvantage attendant on its use is that only one plane of the specimen is in focus at once. In visual work the observer is able by careful and constant manipulation of the fine adjustment to minimise this peculiarity of the objective. In photography, such movement of the objective would result in a blurred image.

In photography with the microscope, a powerful illuminant is desirable. The microscope is either used upright, in which case the

camera is above the microscope; or the arm of the microscope is inclined to a horizontal position, and a camera or other arrangement for holding a photographic plate is adjusted behind it; a usual practice is to insert the eye-piece end of the tube (with or without an eye-piece) into the lens board of the camera, the camera lens being removed. The microscope

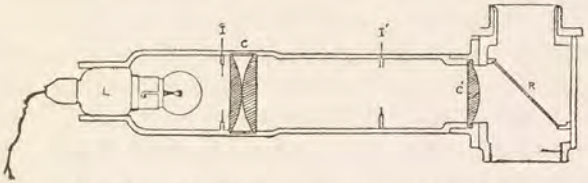


FIG. 30.—VERTICAL ILLUMINATOR WITH LAMP ATTACHED.
The lamp is completely housed. C, C′, Condensing lenses. I, I′, Iris diaphragms.

acts, as it were, as an elaborate lens for the camera. The connection between the two is made light-tight, and the image of the specimen focussed approximately on the ground glass at the back of the camera, whose bellows are extended a greater or lesser distance, according to the size of picture desired. The ground glass is then replaced by a piece of plain glass, whereon delicate detail can be seen with a focussing magnifier, and the sharpest possible

picture obtained by careful use of the fine adjustment. The dark slide then replaces the focussing screen, and the plate is forthwith exposed.

Modern developments in photography have meant much for microscopy. The introduction of plates sensitised to all colours of the spectrum has made possible a choice of almost any wave-length in the spectrum for illuminating the specimen, by the use of carefully standardised colour filters suitable for different light-sources. The old-fashioned state of affairs when red "photographed black" is obsolete, and the photomicrographer can obtain detail or contrast in his pictures at will, by intelligent use of light filters. A knowledge of scientific photography, including colour photography, as opposed to "rule of thumb" practice is now becoming part of the necessary equipment of every accomplished microscopist, and the bond between microscopy and photography draws closer. The photographic research worker relies much on the microscope in his study of sensitive emulsions; while the microscopist asks for plates of finer and finer grain, capable of recording the maximum resolution, and sensitised to light of short wave-length to

record his researches into the microscopical possibilities of invisible light.

Camera and microscope combine not only to produce "still" pictures but records of small organisms in movement. No one who has seen cinematograph films, taken through the microscope, of living protozoa, blood, moving pathogenic organisms, larvæ, etc., can doubt their considerable value for studying the nature of the movement of minute creatures (especially with slow motion), for teaching purposes and educational use generally. It seems strange that more is not heard of such films. As far as academic use is concerned, cost or lack of facilities for exhibition may be the reason. Public exhibition may demand concessions to what is often miscalled "popular taste."

We have said little directly in this book about the educational value of the microscope, being more concerned with the results and methods of microscopical investigation. But there can be no denying its possibilities of service to man in this respect. Few, young or old, can look into a microscope, especially if the specimen is a living one, without natural inquisitiveness prompting the questions, What is this? What about it? Slowly, too slowly,

this is being realised and the microscope is making its way into schools, where in the hands of teachers who are qualified it can do nothing but good.

By way of summary let us set down the great steps that have been made in microscopy.

17th century. The invention of the microscope : *circa* 1600. Thereafter a growing interest in its revelations.

The discovery by Leeuwenhoek of Protozoa and Bacteria—an "invisible world of life," 1675 and 1687.

19th century. The beginnings of histology : stimulated by Bichât, 1771–1801, whose labours brought about a deeper searching into the structure of living things.

The introduction of the achromatic microscope, 1824. With this the names of Goring and Lister in England, Amici in Italy, Selligue, Vincent and Chevalier in France are associated.

The Cell Theory of Schleiden and Schwann, 1838–39; extended by the recognition that all living things are composed of protoplasm (1861).

The application of the microscope to

the study of rock sections by Sorby, 1856 : the beginning of Petrology.

The examination of meteoric iron by Sorby, 1864, which led to the founding of Metallography.

The discovery of the microbic origin of diseases by Pasteur and Koch, 1877.

The demonstration by Van Beneden in 1883, that the chromosomes of the embryo are half-paternal and half-maternal in origin : a historic date in the microscopical study of heredity.

20th century. The great development of industrial microscopy.

The recent progress in the application of new microscopical methods to the investigation of disease.

In conclusion, it may be said that this book only touches the fringe of a vast subject. It may, however, have indicated to the reader what a conspicuous place the microscope holds behind the scenes of present-day civilisation, shown something of its value to man, and last, and not least, have served to suggest that there are more things—on earth at any rate—than some of our philosophies take count of.

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